IncuCyte™ Users Manual

2011A

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1 Warranty

Your Essen BioScience Inc. (Essen) IncuCyte™ product is warranted against defects in material and workmanship for a period of one year following delivery to the Buyer. Essen warrants to its original Buyer only. The Buyer must notify Essen in writing within fifteen (15) days following discovery of the defect. During the warranty period, Essen will, at its option, either repair or replace products that prove to be defective.

For warranty service or repair, this product must be returned to the Essen factory. Buyer shall prepay shipping charges to Essen, and Essen shall pay shipping charges to return the product to the Buyer. However, Buyer shall pay all shipping charges, duties and taxes for products returned to Essen from another country.

1.1 Limitation of Warranty

The foregoing warranty shall not apply to defects resulting from improper or inadequate maintenance by Buyer, Buyer-supplied software or interfacing, unauthorized modification, misuse, or operation outside of the environmental specifications.

NO OTHER WARRANTY IS EXPRESSED OR IMPLIED. ESSEN BIOSCIENCE INC. SPECIFICALLY DISCLAIMS THE IMPLIED WARRANTIES OR MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE.

1.2 Exclusive Remedies

THE REMEDIES PROVIDED HEREIN ARE BUYER'S SOLE AND EXCLUSIVE REMEDIES. ESSEN BIOSCIENCE INC. SHALL NOT BE LIABLE FOR ANY DIRECT, INDIRECT, SPECIAL, INCIDENTAL, OR CONSEQUENTIAL DAMAGES, WHETHER BASED ON CONTRACT, TORT, OR ANY OTHER LEGAL THEORY.

1.3 Warnings and Disclaimers

***Doing any of the following may void your warranty:**

WARNING: DO NOT RUN THE STERILIZATION CYCLE ON AN INCUBATOR WITH THE INCUCYTE™ GANTRY INSIDE.

WARNING: DO NOT TURN OFF THE INCUCYTE™ AND LEAVE THE GANTRY INSIDE THE INCUBATOR. CONDENSATION BUILDUP CAN DAMAGE THE ELECTRONICS.

Essen BioScience is not responsible for data loss due to hardware failure.

2 Getting to know your IncuCyte™

2.1 Introduction

2.1.1 IncuCyte™ Models

Thank you for choosing the IncuCyte™ from Essen BioScience. There are currently 5 models of the IncuCyte™ available:

- The Standard Model IncuCyte™
- The IncuCyte™ EX
- The IncuCyte™ HD
- The IncuCyte™ EX/HD
- The IncuCyte™ FLR/HD

2.1.2 Intended Use

The IncuCyte™ is intended to facilitate live-cell monitoring via customized imaging protocols. It is intended for experimental purposes only. Please read carefully through the operating instructions in this manual to ensure that you can properly and safely utilize all features of your new instrument.

EX

2.1.3 The IncuCyte™ EX

Unlike the Standard Model IncuCyte™, the IncuCyte™ EX microscope is NOT designed to operate within a cell culture incubator. The EX is a special model instrument that is built to interface with an automated cell handling system.

There are some features of the IncuCyte™ software and function that are unique to the IncuCyte™ EX instrument. These special features of the IncuCyte™ EX will appear with an orange background and an icon to the left as demonstrated in this paragraph.

HD

2.1.4 The IncuCyte™ HD Imaging Module

An IncuCyte™ equipped with the HD Imaging Module utilizes a newly developed, unique imaging technique that generates extremely high quality, high definition images of cells. This system introduces a new generation of imaging technology. All cell culture vessels previously compatible with the Standard Model IncuCyte™ are also compatible with the IncuCyte™HD. However, the innovative features of HD imaging also facilitate production of exceptionally high quality images of cells in 96 and 384-well plates. HD images of 96-well plates are superior to those obtained with the Standard Model IncuCyte™, and 384-well plates cannot be imaged in the absence of HD. Some aspects of the IncuCyte™ software are uniquely applicable to HD-enabled units. Aspects of the IncuCyte™ software that are only applicable to the HD-enabled IncuCyte™ will appear with a blue background and an icon to the left as demonstrated in this paragraph.

FLR

2.1.5 The IncuCyte™ FLR

The IncuCyte™ FLR is an HD-enabled instrument that is also equipped with fluorescence scanning capabilities. When vessels are scanned in fluorescence mode, both an HD and a fluorescence image will be generated.

Features of the IncuCyte™ software that are unique to the FLR will be described using text with a green background and an icon to the left as demonstrated in this paragraph.

Additionally, there are some applications specific to the IncuCyte™ FLR. These applications will be described later in the manual (Section 9, IncuCyte™ FLR, page 201).

2.1.6 Special Model IncuCyte™ Instructions

This manual includes general instructions for the Standard Model IncuCyte™ supplemented with additional sections that discuss instructions or conditions specific to the HD-enabled system or to the special models: IncuCyte™ EX and IncuCyte™ FLR. We recommend that the User read all the instructions for the Standard Model IncuCyte™ and then follow up by reading any extra sections that apply to the IncuCyte™ HD or to the special model IncuCytes™.

2.2 Safety has Priority

Please note the following directions for safe and problem-free operation of your IncuCyte™. Read through these operating instructions carefully.

- It is essential to follow the instructions in Section 3, page 13 when putting your new IncuCyte™ into operation.
- The unit must only be connected to a receptacle-outlet with a grounding connection.
- Use only the AC power cord supplied or an equivalent cord with an IEC-320-C13 standard connector approved for operation at 5 amps and the appropriate voltage for your local power. Also, ensure the local line voltage falls within the range printed on the IncuCyte™ Controller.
- NEVER open the IncuCyte™ controller. It does not contain any parts that need to be maintained, repaired or changed by the User.
- If the IncuCyte™ is not used in the manner as described in this manual, provided protections may be impaired.

Your IncuCyte™ is of rugged construction, but it is still a precision instrument. If you treat it with the appropriate care, it will reward you with many years of trouble-free operation. Should you have problems that require service, please contact:

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2.3 Unpacking and Checking the IncuCyte™

The IncuCyte™ System is shipped in two boxes, one containing the automated microscope and the other containing the controller.

NOTE: The IncuCyte™ consists of 2 components: the controller and the microscope. The controller remains outside the cell culture incubator, while the microscope is placed inside the incubator and holds the cells for monitoring. These two components are shipped in separate boxes.

EX

Remember, the IncuCyte™ EX microscope is NOT designed for use in a cell culture incubator.

To unpack the microscope, start by setting the box with the correct side up on the floor or on a sturdy bench top. Start removing materials from the top as shown in the exploded diagram (Figure 2-1). The accessory kit can be removed first through the opening in the top foam piece. The kit contains all of the trays for holding flasks and plates that were ordered with your system.

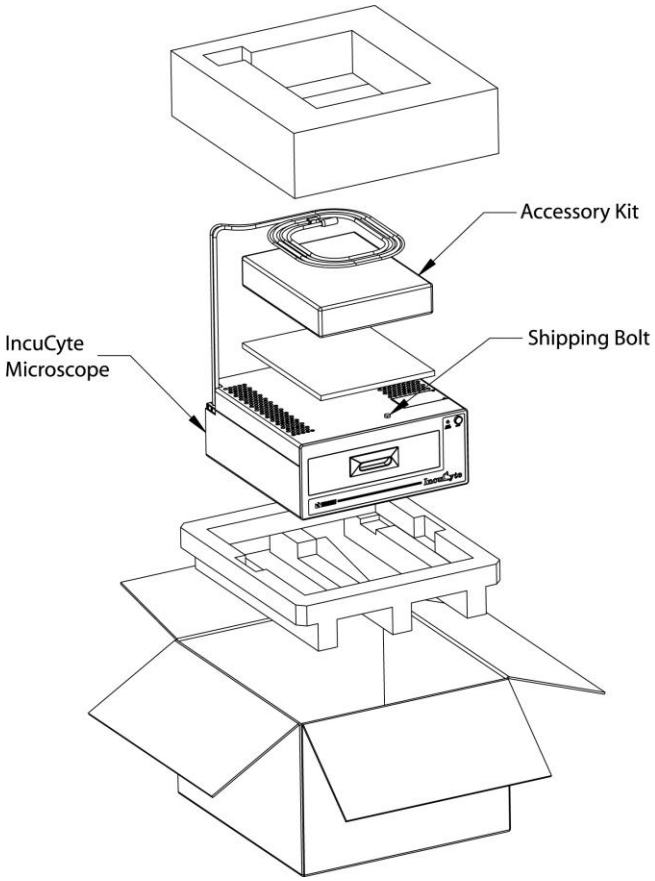


Figure 2-1. IncuCyte™ Microscope Packaging.

The microscope weighs approximately 33 pounds, so it is recommended that one person hold the box while a second carefully removes the microscope from the bottom foam piece and places it on a stable work surface. The unit should then be removed from its plastic bag (not shown in Figure 2-1) and inspected for any obvious signs of external damage.

If the unit appears in good condition, use a wrench to remove the shipping bolt before further inspection and use. After removing the bolt, the drawer on the unit should open and close smoothly. Please inspect the drawer for any signs of damage.

The controller should be removed from its box and bag in a similar fashion (see Figure 2-2). The controller box will contain the controller and, under most circumstances, it will also include a power cord appropriate for your local utilities. However, while Essen provides power cords compatible with most utilities, there will be instances in which Essen does not stock the compatible cord. In these rare circumstances, the purchaser will have to provide their own cord.

IMPORTANT: Use only the AC power cord supplied or an equivalent cord with an IEC-320-C13 standard connector approved for operation at 5 amps and the appropriate voltage for your local power. Also, ensure the local line voltage falls within the range printed on the IncuCyte™ Controller.

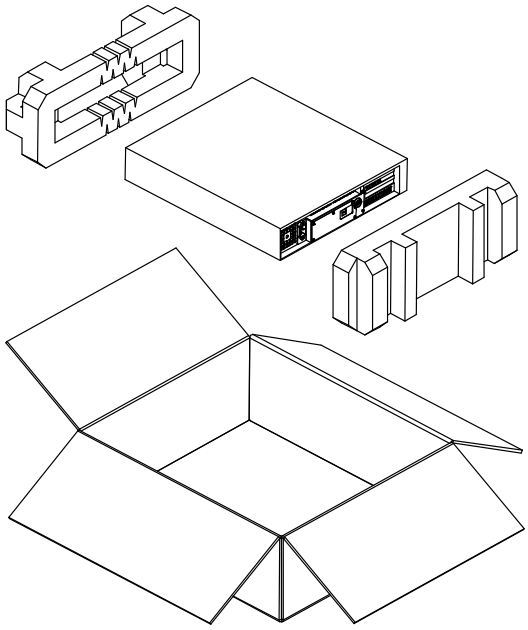


Figure 2-2. IncuCyte™ Controller Packaging.

If the device is damaged in shipping, please call Essen BioScience immediately. You may then be asked to return the unit. When returning an instrument please replace the shipping bolt (Hand Tighten Only!) and pack it in the original containers as shown in Figure 2-1 and Figure 2-2.

EX

2.3.1 IncuCyte™ EX Type C Drawer Brace Removal

The IncuCyte™ EX Type C instrument is shipped with a drawer brace that must be removed after removal of the shipping bolt. See Figure 2-3. Follow the steps below to remove the drawer brace.

With the instrument powered on, press and release the red Stop button on the front panel. The drawer will automatically extend.

Using a number 1 or number 2 Phillips screwdriver, remove the two # 6-32 Phillips pan-head screws indicated in Figure 2-3. The drawer brace is now free to remove as well. Press and release the red Stop button to retract the drawer.

The two screws and drawer brace as well as the shipping bolt should be kept together and not discarded. These items will be required if the instrument should require shipment again at a later date.

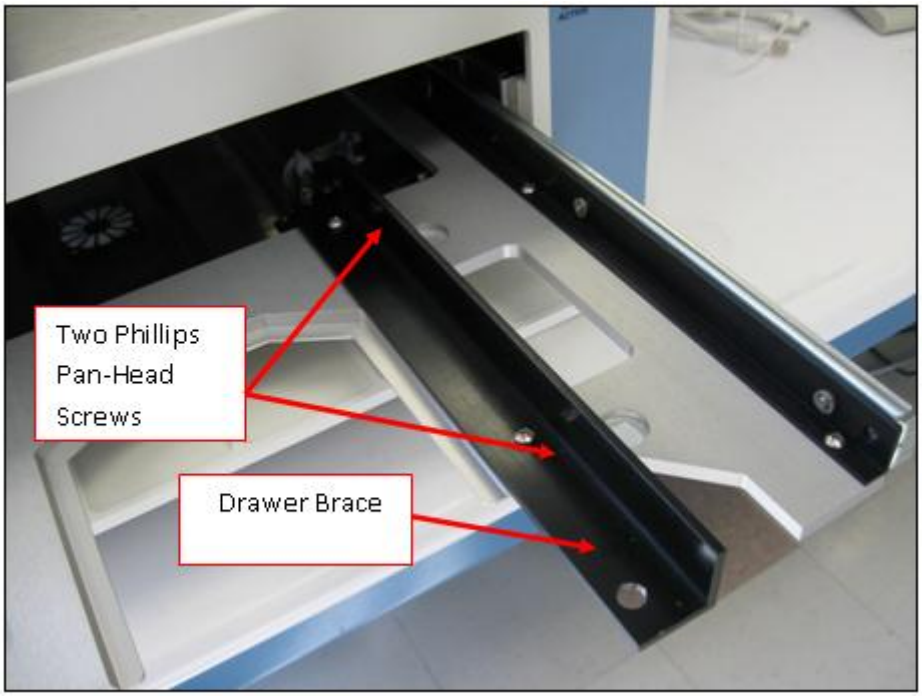


Figure 2-3. Removal of the Drawer Brace

3 Installation of the IncuCyte™ Hardware

3.1 Positioning the Controller

It is important to put the controller into position before placing the microscope into the incubator. The controller should be placed on a level surface within 3 feet of the incubator's cable port. The top of the incubator often works well. Do not, however, connect any of the controller cabling at this point.

3.2 Placing the Microscope into the Incubator

EX

IMPORTANT! The IncuCyte™ EX microscope is NOT designed for use inside a cell culture incubator. It is not compatible with the size, temperature or humidity of an incubator environment. NEVER place the IncuCyte™ EX into a cell culture incubator!

When choosing a shelf for the microscope, keep in mind that flasks and plates are placed into the IncuCyte™ cell drawer from above. For incubators that are at table height, a shelf installed near the bottom of the incubator is usually most convenient. Approximately 8 inches of height will be used by the instrument. In most incubators, this usually allows room for at least two shelves to be installed above the microscope. Remove all shelves above the targeted shelf before setting the microscope into the incubator. Feed the two cables from the microscope out through the cable access port on the back or side of the incubator. The cables can usually be routed up a corner of the incubator so as not to interfere with the installation of shelves above the microscope. A split stopper supplied with the unit can be used to plug the access hole around the cables. Shelves can be reinstalled above the unit as close to the incubators as the mounting brackets allow.

1. WARNING! The top cover of the microscope should NEVER be used as a shelf and should NEVER be used to support a shelf.

2. After the microscope is placed in the incubator, proceed IMMEDIATELY to the next two sections. To avoid large amounts of condensation on the unit, it is desirable to connect and power the unit within a few minutes of installation.

For connecting the cabling, see the rear panel of the IncuCyte™ controller (Figure 3-1). There are two cables that connect the IncuCyte™ controller to the microscope. These cables, which are fixed to the microscope, should be connected to the controller before the unit is powered on. The larger, circular style plug on the microscope main cable should be connected to the mating socket on the back of the controller. The second cable is a FireWire interface to the digital camera in the microscope. This should be plugged into the mating FireWire connector on the rear of the controller. The power cord for the unit can now be installed. Use only the AC power cord supplied or an equivalent cord with an IEC-320-C13 standard connector approved for operation at 5 amps and the appropriate voltage for your local power.

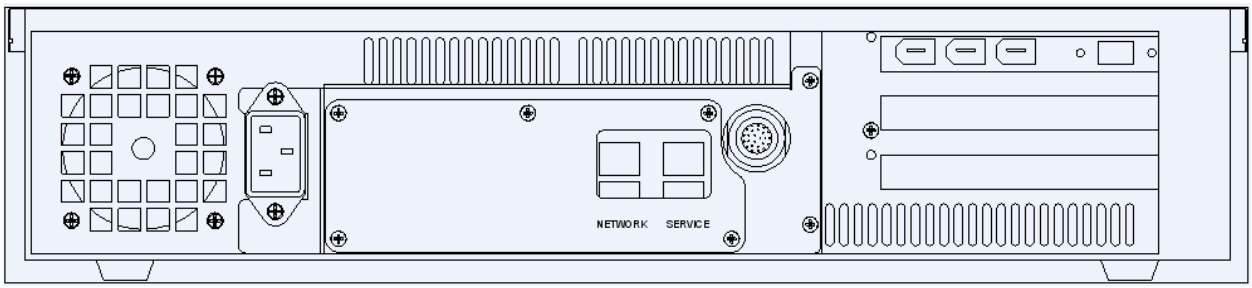


Figure 3-1. Controller Rear Panel

3.3 Switching the IncuCyte™ On and Off

The power switch on the IncuCyte™ can be found under the front panel cover. The system can be started or stopped by quickly depressing and then releasing the power switch. When the system is running, it may take 30 seconds for the system to save any settings and data before it powers down. If the system fails to power down, press and hold the power switch for 10 seconds.

IMPORTANT: The IncuCyte™ should be powered on immediately after installation into the incubator. It is recommended that the unit be powered on at all times when it is in the incubator to minimize the formation of condensation on the system. Proceed to the next Section, Warming up the Microscope, to learn how to warm up the microscope.

3.4 Warming up the Microscope

After the system has been on for 1 minute, a warm-up cycle should be run to get all of the components in the system up to temperature quickly and avoid condensation. This can be done by pressing and holding the Stop button on the front of the microscope for more than 5 seconds. The warm-up cycle will begin after a 5 second pause after the button is released and will continue for approximately 30 minutes. The green activity light on the front of the IncuCyte™ will be lit during the warm-up. If this light does not come on, repeat the press-and-hold sequence.

3.4.1 Auto Warm-up

In the event that the manual warm-up process is omitted (for instance, if the IncuCyte™ is temporarily removed from the incubator, and the person replacing it forgets to run the manual warm-up procedure afterwards), then an auto warm-up process will be initiated. The auto warm-up is NOT intended as a substitute for the manual warm-up cycle but, rather, it is a backup process that occurs in those instances where the manual warm-up is accidentally omitted.

When auto warm-up starts, the microscope is powered on and the front-panel LED lights up until the camera achieves the requisite temperature. If the microscope is placed into the incubator cold, then the auto warm-up process is automatically triggered. Auto-warmup keeps the camera powered on for up to 90

minutes until it reaches a temperature slightly above that of the standard incubator temperature. Auto warm-up cannot occur if the controller is not powered on.

The auto warm-up cycle will be interrupted by any and all device activities, such as a manual warm-up, a device test or a scan. However, auto warm-up will resume if the camera has not achieved its target temperature after the interrupting device activities have been concluded.

3.4.2 IncuCyte™ Power Supply Filter

The filter for the IncuCyte™ controller power supply is located behind the front panel (see Figure 3-2). Although this filter is easily accessible, it is NOT a consumable part, and Users should not attempt to exchange it themselves. If there are any questions about this filter, please contact Essen BioScience for more information.

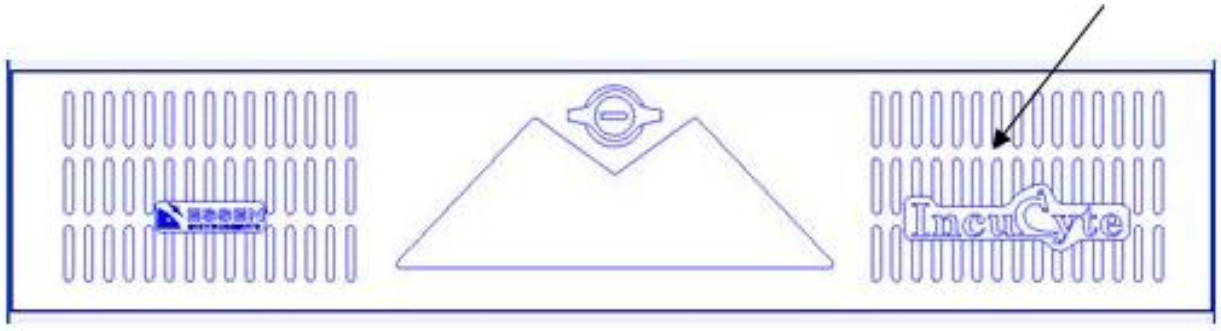


Figure 3-2. IncuCyte™ Front Panel. The Power Supply Filter is located behind the front panel on the side indicated by the arrow.

‘

4 Establishing Network Connections

The IncuCyte™ controller houses an embedded control computer that must be connected to your network in the same way that ordinary PC's running Microsoft Windows are connected. We recommend that you consult with your IT department during the network connection, configuration and verification processes. A standard Ethernet cable is used to connect the controller to a network hub or switch. The unit is equipped with two network ports. The leftmost port is used as the primary network interface to connect the IncuCyte™ controller to the network. The jack on the right is an auxiliary port for servicing the controller. See Figure 3-1 for details.

The controller is configured at the factory for automatic assignment of its IP address on a network using DHCP (Dynamic Host Configuration Protocol). Follow the procedures described in Section 4.1, DHCP Based Networks to connect to this type of network. If the network is not configured to assign IP addresses dynamically, then a static IP address must be assigned to the controller. See Section 4.2 (page 17), Static IP Based Networks, to reconfigure the IncuCyte™ controller for a fixed IP network.

4.1 DHCP Based Networks

Turn off power to the unit, and connect the controller's primary Ethernet port to the network with a standard Ethernet cable. Power on the controller and wait a few minutes before verifying the controller is networked correctly.

If connected correctly, the controller will appear as a computer on the Microsoft Windows Network as a member of the "Workgroup" group of computers. Assume that the controller is assigned the name ICXXXXX in the factory, where XXXXX is the 5-digit serial number of the controller. This serial number is found on a label on the front panel of the controller behind the hinged front plate.

To verify that the ICXXXXX controller has been successfully added to the network, a Windows-based PC (hereby referred to as the "PC") on the same network is required. These verification methods are discussed separately for Windows Vista and Windows XP PC's.

4.1.1 Verifying the IncuCyte™ Controller Network Connection using Windows Vista or Windows 7

1. Under the Windows "Start" menu, select "Network" (Vista) or "Computer" (Windows 7).
    a. *Windows 7 only*: On the left side of the window there is a list of destinations ("Favorites", "Libraries", *etc.*). Look for the "Network" destination and select this by left-clicking.
2. Look for the computer named ICXXXXX (recall that XXXXX is the 5-digit controller serial number). If you find it in the window, then the controller was successfully added to the network, and you are done. If not, you should attempt to find the controller on the network by continuing to the next step.

3. At the top of the window there is a text area that reads "► Network ►". Left-click in an empty area to the right of this text and type the following: \\ICXXXXX followed by the "Enter" key.
4. If the controller is found on the network, you will be prompted for a User name and password. Press the "Cancel" button to exit. If the controller is not found, then a "Cannot Find" message will be displayed after a brief search period.

4.1.2 Verifying the IncuCyte™ Controller Network Connection using Windows XP

1. Open "My Computer" from the PC's Windows Start menu.
2. Under the heading "Other Places" on the left side of the window, open "My Network Places."
3. Under the heading "Network Tasks" on the left side of the window, click once on "View workgroup computers."
4. Look for the computer named ICXXXXX (recall that XXXXX is the 5-digit controller serial number). If you find it in the window, then the controller was successfully added to the network, and you are done. If not, you should attempt to find the controller on the network by continuing to the next step.
5. Verify that the "Address" toolbar is displayed at the top of the window. Under the "View" menu time at the top of the window, verify that the "Toolbars - > Address Bar" menu item has a check mark next to it.
6. Type the following in the "Address" box at the top of the window: \\ICXXXXX.
7. Click on the "Go" button to the right of the address bar. If the controller is found on the network, you will be prompted for a User name and password. Press the "Cancel" button to exit. If the controller is not found, then a "Cannot Find" message will be displayed after a brief search period.

4.2 Static IP Based Networks

In order to reconfigure the IncuCyte™ controller for a static IP address, a direct connection must first be made to the controller. The connection is made to the auxiliary port network interface which ships configured with the following static IP address: 192.168.128.130. To use this port to connect to the controller, a single PC should be connected to the controller box. This can be accomplished by either a direct connection between the auxiliary port and the PC using a crossover Ethernet cable, or by connecting the PC and the controller to a network hub or switch using standard cables (See Figure 4-1). There are two possible configurations of this 2-device network.

NOTE: There must be only 2 devices on this simple network!

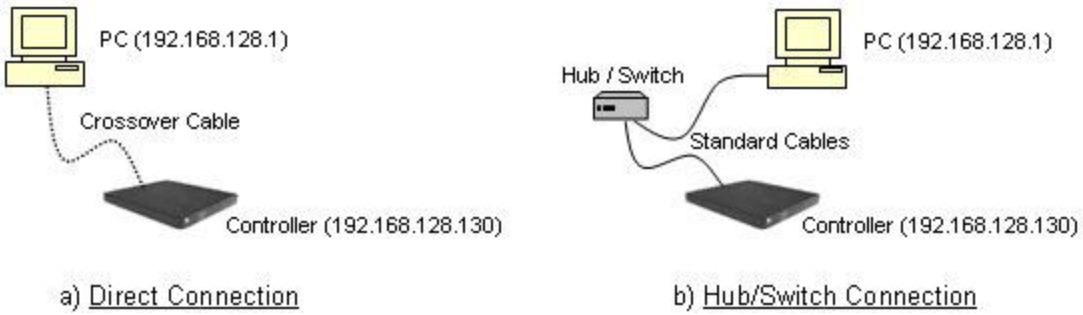


Figure 4-1. Connection to auxiliary controller port. A 2-device network is established between a PC and the controller via either (a) Direct Connection using a crossover Ethernet cable or (b) connection through a hub or switch using standard cables

With both devices powered down, connect the PC and the controller in one of the two configurations shown using the auxiliary network interface on controller (see). After connecting, power up the PC and the controller. The key to establishing a simple 2-device network lies with the correct configuration of the PC's Ethernet port. Like the auxiliary port on the controller, the PC Ethernet port must be configured with a static IP address. The steps for establishing and verifying this network are explained in Section 4.2.1 for Windows.

4.2.1 Verifying the Direct Network Connection using Windows Vista or Windows 7

1. Open the "Control Panel" under the Windows "Start" menu.
2. Select "Network and Internet", then "Network Sharing Center" at the top of the page.
3. In the left pane of the window, select "Manage network connections" under the Tasks List (Vista) or "Change adapter setting" (Windows 7) in the upper portion of the pane.
4. Identify the network connection used in the 2-device network. It will most likely be labeled "Local Area Connection," but could have a different name. Right-click the connection and select "Properties" from the menu.
5. In the new window you will find a box with a list of items used by the connection. Select the "Internet Protocol Version 4 (TCP/IPv4)" item and press the "Properties" button below the list of items.
6. Copy down the settings found under the "General" tab and other tabs. Some of these setting will be modified to create the 2-device network, and you will want to remember the current settings when it comes time to reset the port to its regular configuration.
7. On the "General" tab, select the "Use the following IP address" Radio Button."
8. Type in the following IP address: 192.168.128.1 (Figure 4-1, page 18).

9. Type in the following Subnet mask: 255.255.255.0.
10. Press “OK” to exit the property sheet.
11. Press “OK” again to effect the change to the connection.
12. Follow the steps in Section 4.1.1 (page 16) for verifying the presence of the controller on the network.

4.2.2 Verifying the Direct Network Connection using Windows XP

1. Open “My Computer” from the PC’s Windows Start menu.
2. Under the heading “See also” on the left side of the window, open “Network and Dial-up Connections.”
3. Identify the network connection used in the 2-device network. It will most likely be labeled “Local Area Connection,” but could have a different name. Right-click the connection and select “Properties” from the menu.
4. Under the General tab find the box with a list of items used by the connection. Select the “Internet Protocol (TCP/IP)” item and press the “Properties” button below the list of items.
5. Copy down the settings found under the “General” tab and other tabs. Some of these setting will be modified to create the 2-device network, and you will want to remember the current settings when it comes time to reset the port to its regular configuration.
6. On the “General” tab, select the “Use the following IP address” Radio Button."
7. Type in the following IP address: 192.168.128.1 (Figure 4-1, page 18).
8. Type in the following Subnet mask: 255.255.255.0.
9. Press “OK” to exit the property sheet.
10. Press “OK” again to effect the change to the connection.
11. Follow the steps in Section 4.1.2 (page 18) for verifying the presence of the controller on the network.
12. Follow the steps in Section 4.1.1 (page 16) for verifying the presence of the controller on the network.

Recall that the ultimate goal is to configure the controller’s primary Ethernet port with a static IP address selected for your corporate network. Once the 2-device network has been established and verified, you must connect to the controller’s desktop in order to configure the primary port. Follow the steps in Section 5.4 (page 23), Additional information on creating User accounts can be found in Section 8.6.3 (page 95). Once on the desktop, the controller’s primary Ethernet port can be configured with a static IP address in the same way that the PC’s primary port was configured. Use the Windows XP instructions for the controller configuration. After configuring the primary Ethernet port, log off of the controller’s desktop, dismantle the 2-device network, and reconfigure the PC Ethernet port to its original settings.

5 Installation of the IncuCyte™ Control Software

The IncuCyte™ control software is used to both configure the instrument to collect data and for post-collection viewing and analysis of these data. This software is designed to run on PC's running Windows XP or Windows Vista and requires an Administrator account to install. Insert the IncuCyte™ CD, and the installer should automatically run. If it does not run, open a window to the CD drive and run the "IncuCyteSetup.exe" file.

NOTE: This version of the IncuCyte™ Control Software is not backwards compatible with IncuCyte™ microscopes that are running earlier versions of the software. While this version CAN be used to open data sets archived from earlier versions, it CANNOT communicate with an IncuCyte™ controller that is running earlier software versions. Contact Essen BioScience technical support for instructions for updating controller software.

5.1 IncuCyte™ Control Software Installation using Windows XP

1. Review the license agreement. To continue installation you must Agree.
2. Choose a destination folder and Install.
3. If prompted, install .NET Framework 3.5.
4. Install the Windows Media Player Codecs. (Required to save movies).

NOTE: It is possible you will get a message titled "Windows Media player 10" stating that "This version of Windows Media Technologies is incompatible with this version of windows…." This message can be disregarded. Just click Cancel. The incompatibility message communicates that the machine already has a newer version of Windows Media Codecs (for movie generation). It will be followed by a message entitled "Windows Media Player Codecs Setup" stating that "The Codec package for the 7+ Player has been installed successfully."

5. If prompted, install the Windows Media Encoder. (Required to save movies).
6. Close the installer when completed.

5.2 IncuCyte™ Control Software Installation using Windows Vista and Windows 7

1. Review the license agreement. To continue installation you must Agree.
2. Choose a destination folder and Install.
3. If prompted, install the .NET Framework 3.5.

4. If prompted, install the Windows Media Encoder. (Required to save movies).
5. Close the installer when completed.

5.3 Connecting

5.3.1 Setting the Date and Time

The IncuCyte™ controller settings will be synchronized with the local computer at the time of installation. It is unlikely that any further synchronization will be required after that point. However if, for some reason, synchronization is required at a later date, the IncuCyte™ software will issue a prompt to this effect. The prompt will appear after login but before the software is launched. The exact verbage of the prompt will depend on which settings are in a state of disagreement. Figure 5-1 displays the window that will appear if an individual at the User or Guest permission level logs onto the software and Time Zone synchronization is required. A slightly different message will appear if the individual is at the Administrator permission level.

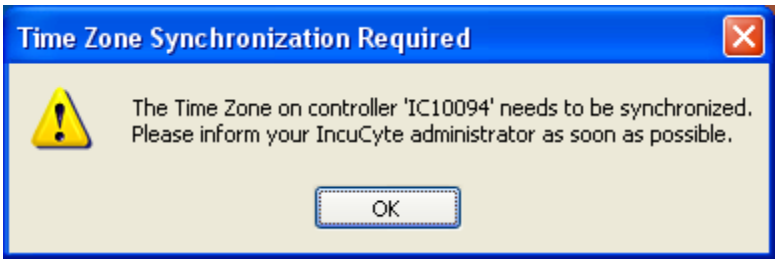

Time Zone Synchronization Required
The Time Zone on controller 'IC10094' needs to be synchronized.
Please inform your IncuCyte administrator as soon as possible.
OK

Figure 5-1. Time Zone Synchronization Window at the User or Guest Permission Level

NOTE:

1. We strongly recommend that ONLY individuals at the Administrator permission level implement changes to the controller time settings.

2. Time or Time Zone synchronization is ONLY required if the IncuCyte™ software issues a prompt, such the example displayed in Figure 5-1.

Detailed instructions regarding synchronization of the controller and local settings can be found in Section 8.6.2, page 90.

5.3.2 Establishing User Accounts

Essen BioScience will establish the initial Administrator account. Additional Users can be created using the Create User function. All IncuCyte™ Users must have an account on each instrument they wish to use. However, we recommend that the number of Users with Administrator level permission be kept to a minimum.

To establish new User accounts, click on the Administer IncuCyte™ task bar in the upper left corner of the main window. The available Administrator functions are organized within a series of tabs. Select the Accounts tab to access password and accounts information.

The Create User function is self-explanatory. The Change Password menu allows Users to set and change their own passwords. This function is available to Users at all permission levels. The Create User and Delete User functions, however, are only available to Users with the Administrator permission level. New Users must have a unique User name. Passwords are case sensitive, and new Users should be encouraged to change their password the first time they log on. When creating a new User, it is important to select an appropriate Permission Level. Users with an Administrator Permission Level have all of the functions of the IncuCyte™ control program available to them. A User with a User Permission Level is able to change his/her password, view device information, configure the device to collect data and perform all post-collection analysis (view, plot, annotate, etc). The "Guest" Permission level is one step below the "User" level in that guests cannot configure the device to collect data. Additional information regarding this topic can be found in Section 8.6.3 (page 95).

It is important to note that there are some differences between the permissible functions of the Standard Model IncuCyte™ and the IncuCyte™ EX.

The software functions available at the various permission levels are summarized in the tables displayed in Figure 5-2 and Figure 5-3.

Function at Permission Level:	Administrator	User	Guest
View/Analyze/Annotate Notes	X	X	X
Change Password	X	X	X
Configure IncuCyte Scans	X	X	
Add/Delete user	X		

Figure 5-2. Permission Level Functions for the Standard Model IncuCyte™, IncuCyte™ HD, and IncuCyte™ FLR.

Function at Permission Level:	Administrator	User	Guest
View/Analyze	X	X	X
Annotate Properties/ Notes			
Change Password	X	X	X
Configure IncuCyte Scans			
Add/Delete user	X		

Figure 5-3. Permission Level Functions for IncuCyte™ EX

EX

Additional information on creating User accounts can be found in Section 8.6.3 (page 95).

5.4 Remote Administration of the Controller

The IncuCyte ™ controller consists of a single-board computer running a Windows-based embedded operating system. This computer is dedicated to controlling the IncuCyte™ hardware and providing remote access to the data collected by the instrument. To insure reliable and proper operation of your IncuCyte™, the factory settings of the controller operating system should not be altered. However, we recognize that there may be instances when it is desired or even required to log onto the embedded controller desktop. Some examples include:

1. Renaming the controller from the factory-set name
2. Configuring the primary Ethernet port with a static IP address
3. Installing anti-virus and/or firewall software
4. Installing Essen-recommended Windows updates

The IncuCyte™ controller has been shipped with a unique Administrator password for accessing the controller's Windows desktop. Essen BioScience maintains a record of this password, so we strongly discourage you from renaming the Administrator password. We also recommend that this password be protected and that the controller operating system be accessed only out of necessity. Altering the controller operating system as little as possible is the best way to guarantee stability of the software that controls the IncuCyte™.

The easiest method for accessing the controller desktop is through the Windows "Remote Desktop Connection" tool. Windows XP Users can access this utility through the Windows Start menu under the "Programs -> Accessories -> Communications" menu. Users of Windows Vista will find the utility in the "All "Programs►Accessories" menu. For other Windows operating systems, this tool is available as a free download from Microsoft at the following URL:

http://www.microsoft.com/windowsxp/downloads/tools/rdclientdl.mspx

Run the "Remote Desktop Connection" software and type in the name of the IncuCyte™ controller in the "Computer" edit box. If connecting for the very first time, the name used will be the factory-set name. Make sure that you have connected and verified that the controller is on the network (see Sections: 4.1.1[page 16] and 4.1.2 [page 17]). Press the "Connect" button and wait for a Windows login prompt. It may take as long as 15 seconds before the prompt appears. Log into the controller with a User name "Administrator" and the Essen-provided password. The controller operating system should contain the necessary components required to rename the computer, configure the primary Ethernet port, install security software, etc. Please consult with your IT department for answers to questions related to your local network configuration and security software.

IMPORTANT: Always use the “Disconnect” option and not the “Log Off” option when terminating the Remote Desktop Connection via the controller Start menu.

6 Preparing for Cell Culture Monitoring

Now, once the IncuCyte™ is fully installed, connected, and User accounts have been established, you're almost ready to start cell culture monitoring. Just a few remaining points need to be covered before we can get to this final step. These include:

- How to select and place trays into the IncuCyte™
- How to add flasks, plates or dishes to the trays
- And, importantly, how to calibrate the instrument

6.1 Selecting and Placing Trays and Vessels into the IncuCyte™

6.1.1 Selecting Trays

Tissue culture vessels are held within the IncuCyte™ drawer in trays. The IncuCyte™ drawer can hold a maximum of three trays, and each tray will hold different numbers of vessels depending on the tray type.

Vessel: A vessel is a general term used throughout this manual that refers to a cell culture flask, plate or dish.

A table of all available trays and compatible vessels is listed in the Appendix at the end of this manual. This same list can also be accessed through the IncuCyte™ software by selecting Find from the Tray Type pull-down menu in the Schedule Upcoming Scans screen (see Section 8.5.1, page 61). The IncuCyte™ can be configured with any combination of trays and vessels from this list. While the table in the Appendix contains all vessel types and trays available at the time this software version was released, it is likely that new vessels and trays will be added to the list as they appear on the market and as customer demand dictates. These additions can be downloaded into the IncuCyte™ vessel database. See Section 8.6.6 (page 106) regarding the procedure for updating the vessel database.

In order to further facilitate tray selection, each tray will have its Tray Type number stamped on the bottom portion of the tray. Additionally, the shape of each tray will be displayed when it is selected within the software. See Figure 6-1. For more information, see Section 8.5.1, Physical Layout (page 61).

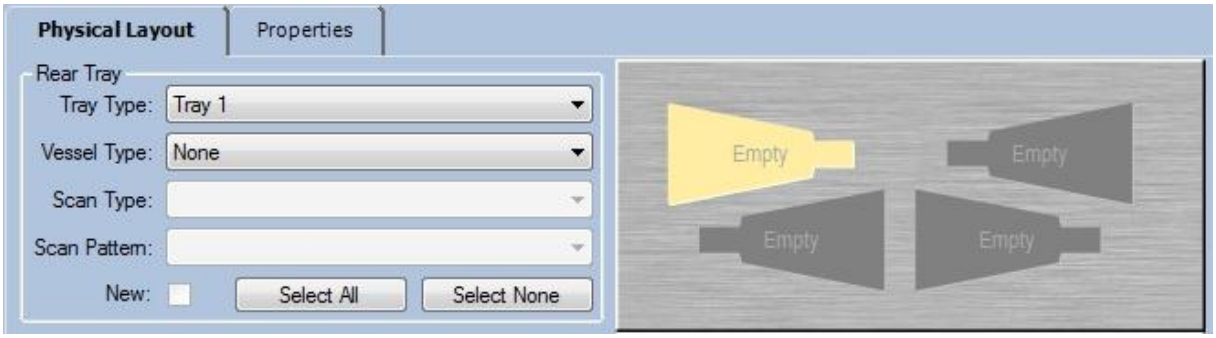


Figure 6-1. Tray Type 1: The physical appearance of Tray Type 1 displayed within the IncuCyte™ software (Schedule Upcoming Scans screen). The four spaces for T-25 flasks are indicated as Empty.

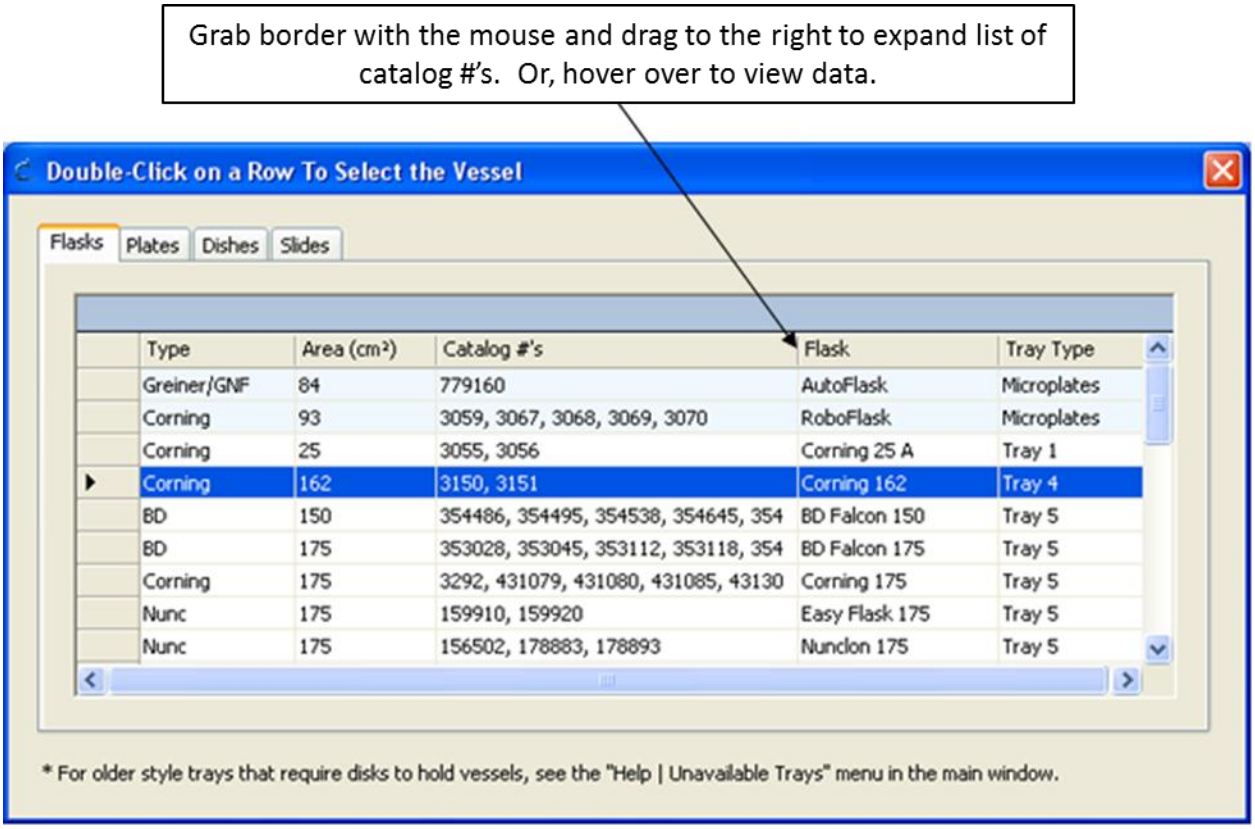

Grab border with the mouse and drag to the right to expand list of catalog #'s. Or, hover over to view data.
Double-Click on a Row To Select the Vessel
Flasks
Plates
Dishes
Slides
Type
Area (cm²)
Catalog #'s
Flask
Tray Type
Greiner/GNF 84 779160 AutoFlask Microplates
Corning 93 3059, 3067, 3068, 3069, 3070 RoboFlask Microplates
Corning 25 3055, 3056 Corning 25 A Tray 1
Corning 162 3150, 3151 Corning 162 Tray 4
BD 150 354486, 354495, 354538, 354645, 354 BD Falcon 150 Tray 5
BD 175 353028, 353045, 353112, 353118, 354 BD Falcon 175 Tray 5
Corning 175 3292, 431079, 431080, 431085, 43130 Corning 175 Tray 5
Nunc 175 159910, 159920 Easy Flask 175 Tray 5
Nunc 175 156502, 178883, 178893 Nunclon 175 Tray 5
* For older style trays that require disks to hold vessels, see the "Help | Unavailable Trays" menu in the main window.

Figure 6-2. Select Find under the Tray Type pull-down menu to obtain a complete list of compatible vessels and their corresponding Tray Types. The example above indicates that the Corning® 162cm^2 flasks with catalog numbers 3150 or 3151 require Tray Type 4. In some cases, all the catalog numbers for a particular vessel type will not be visible at one time. This is the case for the BD 150cm2 vessel. To view the remaining catalog numbers, place the mouse at the Catalog #'s/Flask title border and drag to the right.

6.1.2 Selecting Trays for the IncuCyte™ EX

The IncuCyte™ EX is equipped with a single tray that is built into the instrument. Therefore, it is not necessary to select trays for the IncuCyte™ EX.

6.1.3 Placing Trays into the IncuCyte™

All trays need to be placed into the IncuCyte™ drawer in the proper orientation. To ensure proper orientation, each tray contains three Alignment Windows at its outer perimeter: one top left, one top right, one bottom right. In Figure 6-3 the Alignment Windows are indicated by arrows.

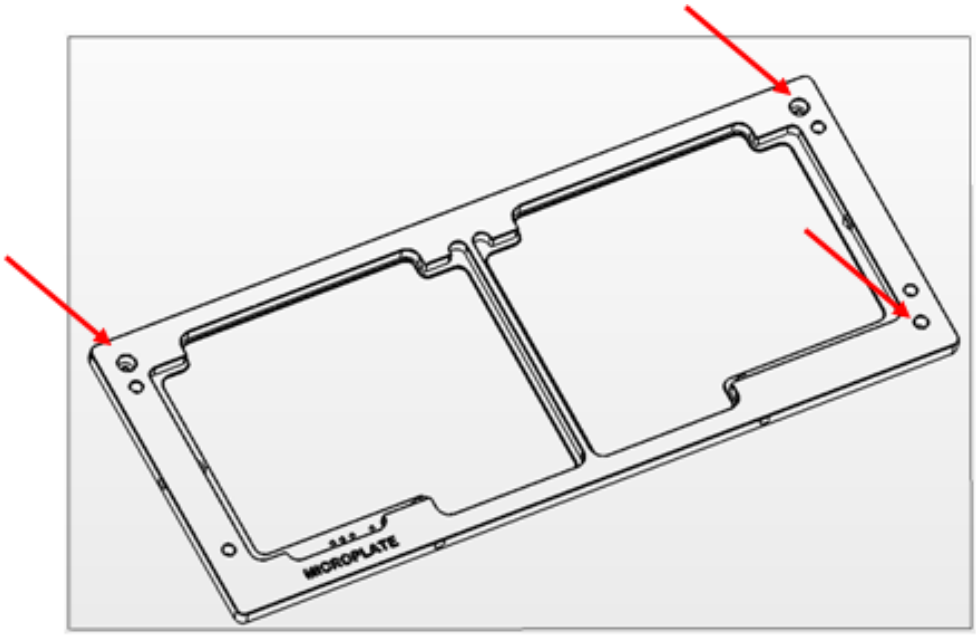


Figure 6-3. Microplate Tray Indicating Location of Alignment Windows

EX

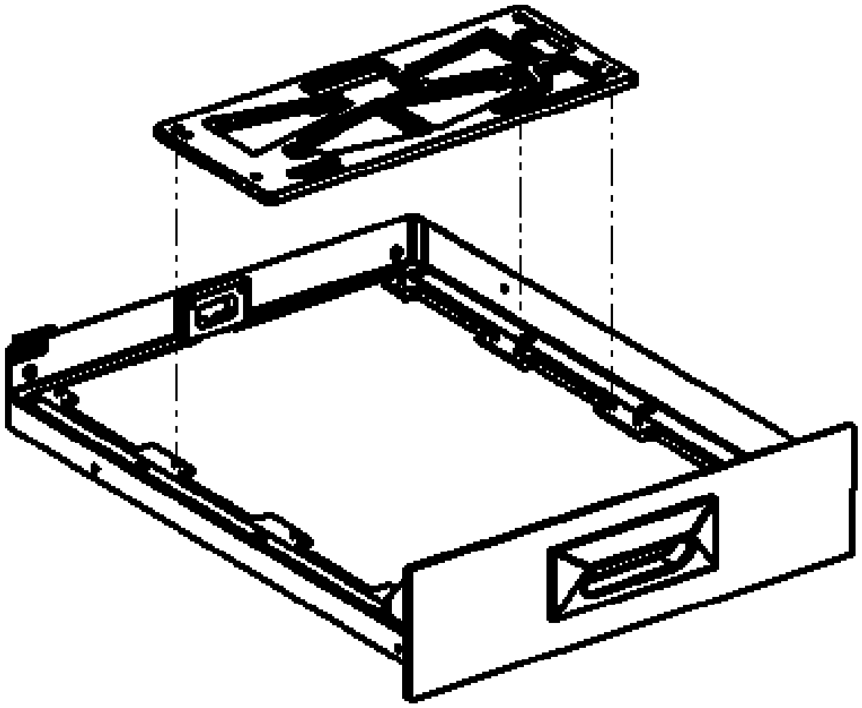


Figure 6-4. Location of Alignment Posts on the Drawer. These posts must be placed through the Alignment Windows as shown.

The 3 tray Alignment Windows fit over 3 posts in the corresponding locations within the IncuCyte™ drawer. When placing trays into the drawer, make sure that the drawer Alignment Posts pass through the tray Alignment Windows. The tray should sit very flat, and the posts should prevent any lateral movement. See Figure 6-4.

EX

6.1.4 Placing Trays into the IncuCyte™ EX

The EX is equipped with a single tray that is built into the instrument. Therefore, it is not necessary to place trays into the IncuCyte™ EX .

6.1.5 Placing Vessels into the IncuCyte™

After the selected trays have been placed into the IncuCyte™ drawer, it is time to add the desired vessels . Be sure the selected vessels sit completely flat within the tray. If the flask is not seated flatly, image quality will be compromised.

Some trays are designed to hold multiple versions of the same vessel (e.g. the same flask size made by different manufacturers). Therefore, the fit of certain vessels within certain trays might not be snug around the edges. Nonetheless, the fit should be adequate for IncuCyte™ function.

The quality of images and subsequent confluence measurements obtained using the IncuCyte™ depend on having a clear, unobstructed light path through the

particular well or flask. Therefore, before use, carefully inspect the vessels selected for the experiment. The following is a list of important issues to avoid:

- Vessels that have scratches on the tops (lids for microplates/dishes) or on the bottoms.
- Drops of media or other liquid on the outside tops/lids of vessels. If this occurs, carefully clean with ethanol.
- Media hanging on the inside of tops/lids of vessels.
- Condensation on top/outside of vessel.
- Touching either the tops or bottoms of vessels during manipulations. Hold vessels by the sides.
- Writing labels on the tops of vessels. The writing will interfere with the images. Label vessels on the sides, or on the very edges of the tops where imaging will not occur.
- It is VERY IMPORTANT that all lids are tightly fastened onto the flasks. If the lids are loose, it can cause the flasks to become displaced within the tray.
- Be sure all vessels are sitting COMPLETELY flat in the trays.

6.2 System Calibration

Before using the IncuCyte™ for the first time, a calibration procedure must be performed. This procedure ensures that the motion of the IncuCyte™ microscope is accurate and repeatable such that images are obtained within their proper, target locations. This is especially important when acquiring images in 96 and 384-well plates. The calibration procedure requires up to 1 hour, and it utilizes a special calibration tray that is shipped out with the unit. The calibration tray is the same size and is inserted in the same fashion as the regular trays (See Section 6.1.3, page 27) for proper insertion of trays into the IncuCyte™). Each of the three tray positions (front, middle and rear) must be calibrated individually.

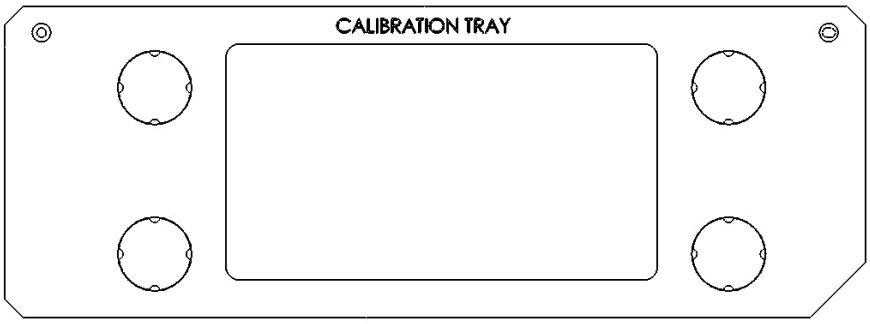


Figure 6-5. The Calibration Tray

What follows is an overview of the calibration procedure. A more detailed explanation of the procedure can be found in Section 8.6.5, page 97. For now, See Figure 6-6.

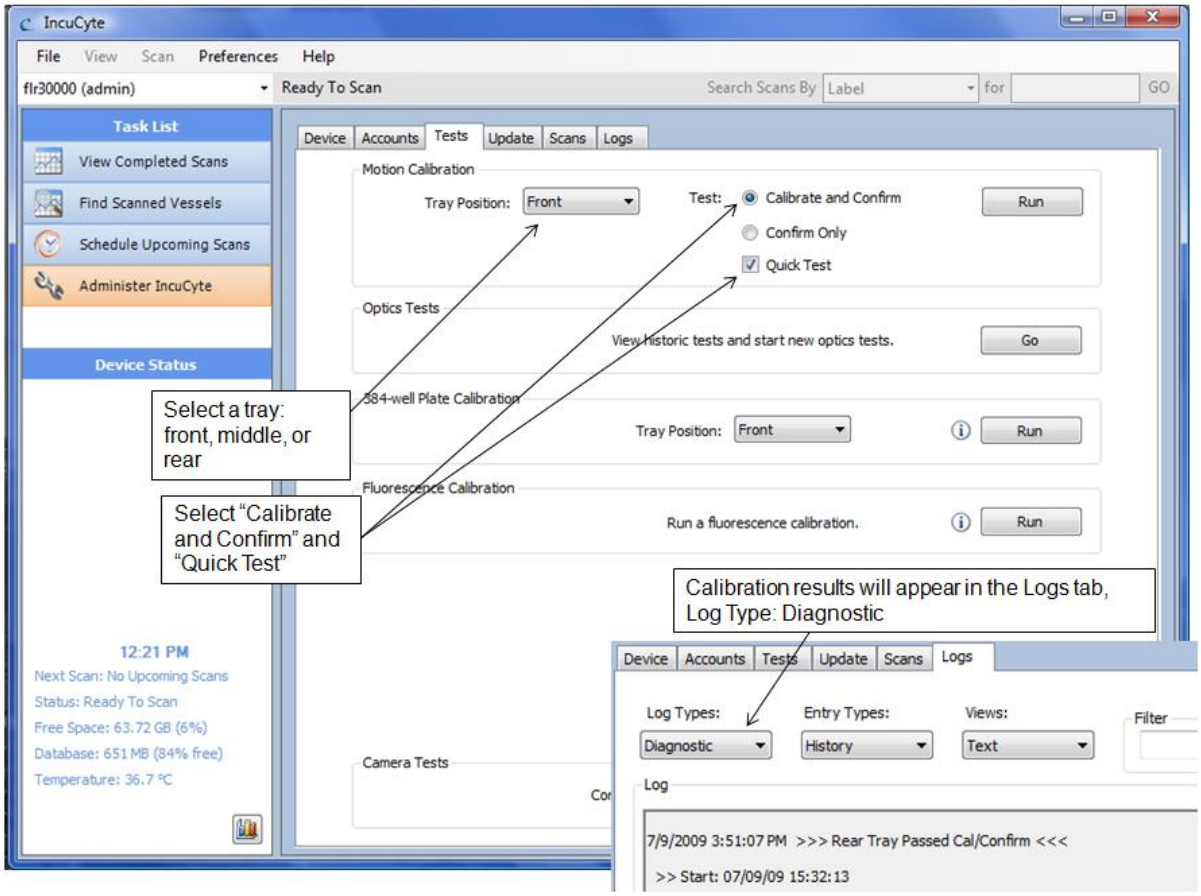


Figure 6-6. Calibration

NOTE: Calibration can ONLY be performed by a User at the Administrator Permission level.

Place the Calibration Tray into one of the tray positions (the order in which tray positions are calibrated is not important, but all 3 must be calibrated).

Navigate to the Tests tab under the Administer IncuCyte™ task bar. The Front, Middle and Rear tray positions can be selected using the pull-down menu. Each Tray Position must be individually calibrated and confirmed.

The first time the instrument is calibrated, run the Calibrate and Confirm Quick Test. See Section 8.6.5, page 97.

The Results of the Calibration and Confirm test can be viewed in the Logs tab. Select Logs, and then use the pull-down menu under Log Type to select

Diagnostic. The calibration results will be displayed in the large window below. If no results are visible, press the Refresh button at the bottom of the screen to update the log. The results will be displayed as coordinate values. The Log will indicate whether the test passed or failed. Check to be sure the test passed for all three tray positions before proceeding to Section 7 (page 35). If the calibration was not successful, cell culture monitoring will not produce quality results.

HD

6.3 Calibration of the IncuCyte™ HD

The standard calibration procedure previously described in Section 6.2 is adequate for scanning all vessels in the IncuCyte™ HD with the important exception of 384-well plates. Because scanning of 384-well plates requires such a high degree of precision, it also requires a special calibration procedure. All three IncuCyte™ tray positions (front, middle, rear) must be individually calibrated before scanning of 384-well plates is initiated.

In order to calibrate the IncuCyte™ for 384-well plate scanning, complete the standard calibration procedure as described in Section 6.2. The additional 384-well Plate Calibration requires a single microplate tray and two EMPTY clear-bottom, black-walled or white-walled, 384-well plates with square wells. Figure 6-7 displays a list of 384-well plates that have been tested and approved for 384-well Plate Calibration:

Manufacturer:	Wall Color:	Catalogue Number:
BD Biosciences	Black	353962
Corning	Black	3712
Greiner	Black	781091
Greiner-GNF	Black	789070-G
Matrix	Black	4332
	White	4330
Nunc	Black	142761
Porvair	Black	312003
	White	311003

Figure 6-7. 384-Well Plates Approved for HD Calibration

We recommend that customers select the same plate type for calibration that they also plan to use most frequently for scanning. If the desired plate type is not among our approved list of candidates, then select the plate type that most closely resembles your plate of interest. Clear-walled plates CANNOT be used for 384-well Plate Calibration, so in that case select the approved plate from the same manufacturer.

It is helpful to pre-warm plates prior to using them for calibration. If room temperature plates are placed directly into the warm incubator, condensation can form on the bottoms of the plates and compromise the calibration results.

ONLY clear bottom, COMPLETELY EMPTY, opaque-walled 384-well plates with square wells from the approved list should be used for the 384-well Plate Calibration procedure.

See Figure 6-8. Follow these steps to run the 384-well plate calibration procedure:

Put two (2) empty 384-well plates into a microplate tray (both spaces in the microplate tray must be occupied).

Select the appropriate tray position in the software – all three tray positions need to be calibrated.

Press Run. The calibration for each tray position takes only a few minutes.

Repeat for all three tray positions.

Figure 6-8. 384-well Plate Calibration

The results for the calibration can be viewed under the Logs tab, Log Type Diagnostic. Unless there is an error message in the log file, the calibration was successful.

EX

6.4 **Calibration** EX

The IncuCyte™ EX does not require a special calibration tray. The built-in vessel tray also serves as the calibration tray. To calibrate the EX, select the Tests tab under the Administer IncuCyte™ task bar. Calibration is identical to that of the Standard Model IncuCyte™ except that the Tray Position cannot be selected (because the EX contains only a single tray). View the calibration results in the Logs tab under Log Type: Diagnostic.

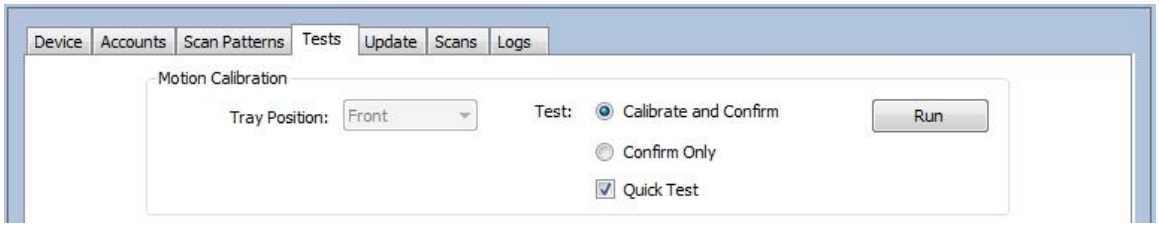


Figure 6-9. Calibrate Device EX

FLR

6.5 **Calibration FLR**

Because all IncuCyte™ FLR's are HD-enabled instruments, they must undergo standard tray calibration as well as 384-well calibration. Additionally, the fluorescent IncuCyte™ requires a special fluorescence calibration procedure.

Tech Note

See the Essen BioScience IncuCyte™ FLR Technical Note entitled, “Using the IncuCyte™ Calibration Kit” for more information.

A calibration kit will be provided with the purchase of the IncuCyte™ FLR, and the instrument will be calibrated at the time of installation. It is currently recommended that the instrument be recalibrated approximately twice a year; however, if the User suspects a drift in the calibration signal, then the system can re-calibrated at any time.

The fluorescence calibration kit includes liquid sufficient for multiple calibrations as well as three calibration slides. These calibration slides are disposable and should be used only once. New calibration kits can be purchased from Essen BioScience. See Section 13, IncuCyte™ Catalog, page 244.

Follow these steps to perform the fluorescence calibration:

Place a microslide tray in the front position of the IncuCyte™. Fluorescence calibration requires use of the front tray position, and the remaining positions can be occupied with trays at the time of calibration.

Load a calibration slide with supplied dye. Use 40µl dye per slide. Place the pipet tip at one end of the slide window and expel the liquid dye. The liquid will wick into the slide window. After all the dye has been expelled, use the pipet to transfer a small amount of dye to the other end of the window to be sure it is completely filled. Avoid getting any dry spots in the window as this will interfere with proper calibration.

Place the calibration slide in the FAR LEFT CUTOUT POSITION of the microslide tray. The orientation of the slide within the cutout position is not important. However, it is ESSENTIAL that the calibration slide is the ONLY slide in the microslide tray. Verify that the rest of the tray is empty.

Select the Tests tab under the Administer IncuCyte™ tasks bar. Then, select the Fluorescence Calibration test. Press the Run button. A new window will open to confirm that the IncuCyte™ is properly configured to run the test. Press the Yes button to start the calibration. See Figure 6-10.

The calibration procedure will begin. Fluorescence Calibration requires ~ 15 minutes. Calibration status will be indicated at the top of the IncuCyte™ software window and in the status box at the bottom left.

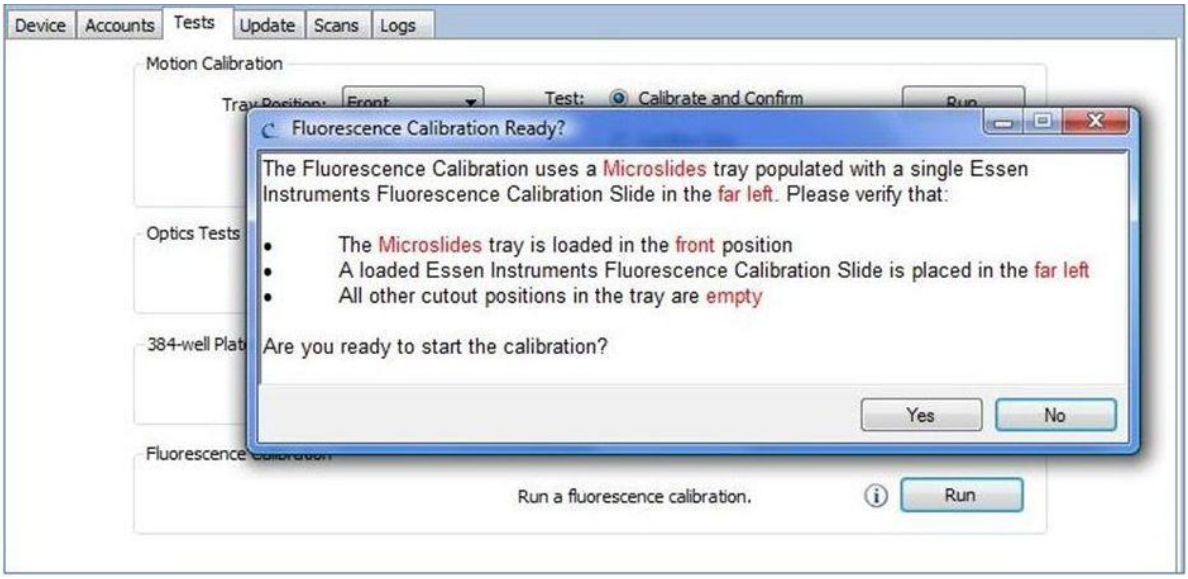


Figure 6-10. Fluorescence Calibration

FLR Calibration Results

When calibration is complete, the results can be viewed under the Logs tab, Log Type: Diagnostic. The log will state whether or not the calibration passed. If the calibration failed, the log will include recommendations to avoid potential issues the next time through.

7 Overview of Cell Culture Monitoring

The following is a general overview of cell culture monitoring. A more detailed explanation of all of this material can be found in Section 8 (page 56), Software Reference.

7.1 Scheduling Scans

So, finally, everything is ready to go. You have selected the trays and vessels that you want and arranged them within the IncuCyte™ drawers. You have checked that all vessels are clean, fit snugly and lay flat within their trays. Now it's time to use the IncuCyte™ software to schedule the desired scans within the 24-hour Repeating Schedule. To get started, select the Schedule Upcoming Scans task bar. This will bring up the corresponding Schedule Upcoming Scans screen (Figure 7-2, page 37). Make sure that the vertical 24-Hour Repeating tab has been selected. The following parameters need to be set in order to set up a scan:

7.1.1 The Scan Active Light and Stop Button

Scan Active Light

Note the green Scan Active light on the upper right corner of the IncuCyte™ microscope. This light will be on if a scan is in progress. It will also be lit under some other circumstances including the IncuCyte™ Self-Test (Section 8.10.6, page 199), and other tests such as the Camera Test or Optics Test. When the Scan Active Light is on, the IncuCyte™ tray drawer will be locked shut. Do NOT try to force the door open while the light is on. To interrupt any ongoing IncuCyte™ activity, press the Stop button. See below.

The Stop Button

The Stop button is located on the front of the IncuCyte™ microscope next to the Scan Active light. Press the Stop Button to interrupt any ongoing IncuCyte™ activity. The most common reason to press the Stop button will be to interrupt an ongoing scan and open the drawer. If a scan is ongoing and the Stop button is pressed, the camera will terminate scanning and return to its “home” position. This homing process will result in a short delay between pushing the button and drawer accessibility. If you want to interrupt the scan and open the drawer, press the Stop button, wait for the microscope to “home,” and then open the drawer when the light turns off. The light turning off indicates that the drawer is now unlocked and can be opened.

EX

The Stop Button EX

The front drawer of the EX CANNOT be opened manually like on the Standard Model IncuCyte™. To open the front drawer on the EX press the Stop button briefly and then release it. Again, there will be a short delay before the door opens. Be patient, and do not press the Stop button and hold it down continuously.

7.1.2 Physical Layout: Select Tray Type, Vessel Type, Scan Pattern, and Scan Type

EX

It is NOT possible to schedule scans on the IncuCyte™ EX using the IncuCyte™ software. The only aspect of scan selection available to EX Users is the selection and creation of scan patterns. For more information on this function, see Section 8.5.4, page 70.

Scan Pattern: The scan pattern describes the number and locations of images that are generated for each well (microplates) or sector (flask/dish). The Scan Pattern is displayed within the software as a pattern of green dots, where each dot represents one image.

Sector: In microplates, the scan pattern is defined as the number of images per well. However, because flasks and dishes don't contain wells, they are artificially divided into units called Sectors. In flasks and dishes, the scan pattern is defined as the number and locations of images for each Sector.

Use the Tray Type pull-down menu to select a Tray Type. After a Tray Type has been selected, highlight one or more empty vessel spaces within the tray.

The Vessel Type pull-down menu below will now become active. Use the Vessel Type pull-down menu to select a vessel.

- Once a vessel has been selected, the Scan Type and Scan Pattern menus will become active. For information on scan types, see Section 7.1.5 (page 42), Section 7.1.5 (page 42) and Setting Scans in the IncuCyte™ FLR (page 37).

Use the Scan Type pull-down menu to choose Phase-Contrast or Fluorescence & Phase-Contrast (see Section 8.5.1 Adding Vessels to)

Use the Scan Pattern menu to select a Scan Pattern. When the software is first launched, the only scan pattern available will be the sample pattern. To create new scan patterns, see Section 8.5.3, Setting Scan Patterns (page 67).

Select the Properties Tab. Enter the properties of each vessel. These include:

- Vessel Label
- Cell Type
- Cell Passage
- Any relevant notes (e.g. concentration at which cells were seeded, etc.).

The first time a scan is scheduled, all vessels entered will be new, so the New box will be inactive (Figure 7-2). Later, however, a new scan might be set up using the same vessel types in the same positions that were used in the previous scan(s). In this case, the New box will be active. Check the New box and then enter the new properties for the new vessel(s). To clear all vessel properties, select the Clear Vessel Properties button.

It is VERY IMPORTANT to always check the New box when a new vessel is scanned. Otherwise, all the scans from the previous vessel will be included with the data from the new vessel (Figure 7-1). If you forget to check the New box, see Section 8.7.9, Change Start. (page 155). Failure to check the New box appropriately will also interfere with searches under the Find Scanned Vessels task bar.

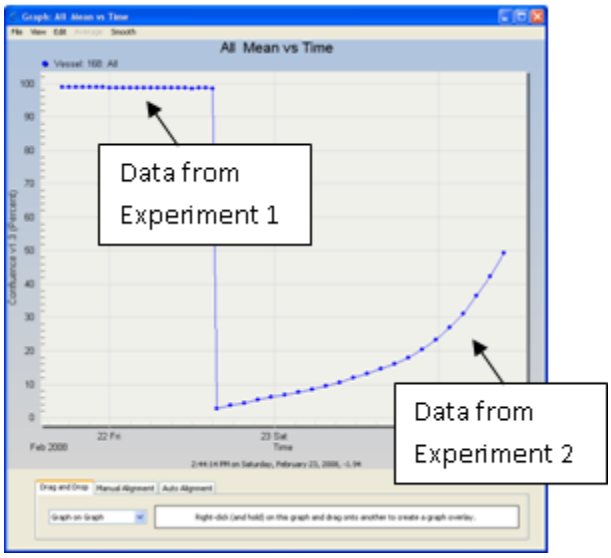


Figure 7-1. The New box was not checked at the beginning of scans for a new vessel (Experiment 1). Therefore, when the flask is graphed, the graph for the previous vessel is connected to the graph for the new vessel (Experiment 2).

FLR

Setting Scans in the IncuCyte™ FLR

Two scan Types are available in the IncuCyte™ FLR: Phase-Contrast or Fluorescence & Phase-Contrast

If Fluorescence & Phase-Contrast is selected, the vessel will be scanned both fluorescently and in HD mode.

- Fast Fluorescence: Allows for faster scanning and analysis with no loss in sensitivity (See Section 8.5.2: Setting Vessel Properties FLR).
- Standard

If Phase-Contrast is selected, the vessel will only be scanned in HD mode.

7.1.3 Set Scan Time(s)

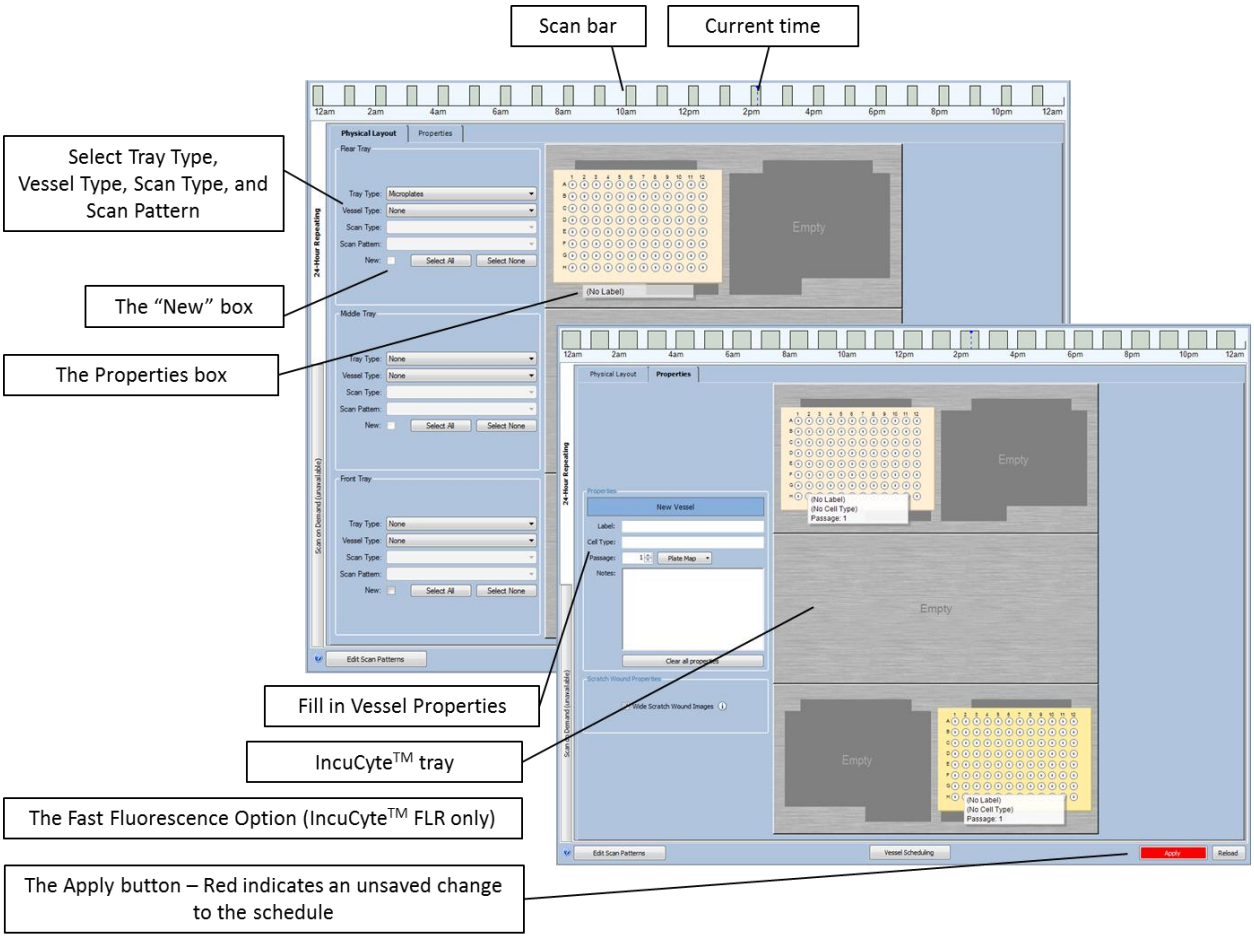

Scan bar
Current time
Select Tray Type, Vessel Type, Scan Type, and Scan Pattern
The "New" box
The Properties box
Fill in Vessel Properties
IncuCyte™ tray
The Fast Fluorescence Option (IncuCyte™ FLR only)
The Apply button – Red indicates an unsaved change to the schedule

Figure 7-2. Schedule Upcoming Scans Screen. First, select Physical Layout, then the Properties, and last, set Scan Times using the Timeline

Look at the Timeline at the top of the IncuCyte™ screen with the vertical 24-Hour Repeating tab selected. The current time will be displayed as a dotted blue line (~2:20pm in Figure 7-2).

Left-click with the mouse on the Timeline to set a scan start time. This will generate a gray Scan Bar. Hold the mouse over the Scan Bar to display the exact scan start time and scan length. Right-click on the bar to adjust the start times by small increments. Left-click and drag the bar to move the start time by large increments. To set multiple scans, create multiple scan bars individually, or use the right-click Scan Bar menu to select a Scan Interval (Scan Bars will appear for each scan time). Use the right-click Scan Bar menu to delete a single scan or all scans. To delete a scan, you may also left-click and drag the Scan Bar off the timeline.

The Timeline represents a 24-hour Repeating time period. Once all the scans for the first 24-hour period have been completed, the IncuCyte™ will automatically

start over for the next 24-hour period. This pattern will repeat until scanning is terminated by the User.

Whenever a scan is applied to 96-well plates, a pop-up window will appear letting the User know that the first scan will be longer by __ minutes (the exact number of minutes depending on the specific scan length). In order for the scan to proceed, the OK button must be selected, confirming that the User is aware of the increased scan time. This longer initial scan length ONLY occurs with 96-well plates. See Section 8.5.7, Scan Length, for more information on this topic.

HD

Note that the longer initial scan time for 96-well plates does NOT apply to the IncuCyte™ HD or the IncuCtye™ FLR.

Vessel Scheduling

When scanning multiple vessels, the Vessel Scheduling button at the bottom of the Schedule Upcoming Scans screen allows the User to insert cooling (idle) times between vessel scans and customize the order in which IncuCyte™ scans vessels. Using the Auto-Cool function is recommended and allows for the most efficient vessel scheduling. For a more detailed view, see Section 8.5.8 Vessel Scheduling**.**

When the Physical Layout, Properties and Scan Time(s) have been set, select the Apply button at the bottom of the screen. A window will appear confirming that scanning parameters have been applied. To terminate scanning, delete all the Scan Bars and then hit Apply. A scan can also be manually terminated by pushing the Stop button on the front of the IncuCyte™. Pushing the Stop button will abort the scan that is ongoing at that time. However, if scanning is terminated in this way, the IncuCyte™ will start scanning again at the next scheduled scan time. The ONLY way to terminate all future scans is by deleting all the Scan Bars and then selecting Apply.

IMPORTANT:

1. The scanning process causes the IncuCyte™ to become warm. In order to prevent overheating, it is recommended that total scan time not exceed 45 minutes.

2. Scan times CANNOT overlap. If attempting to set scan times that overlap, the IncuCyte™ will generate an error message.

3. The Apply button MUST be selected before new parameters will be saved and scanning will be initiated.

Further reading on this topic is recommended. See Section 8.5.7, page 76.

7.1.4 Scan on Demand

In addition to the 24-Hour Repeating scan schedule, users also have the ability to scan in the Scan on Demand mode. Simplistically, this means that the IncuCyte™ can be used to scan individual vessels at times when the IncuCyte™ is not scanning. This includes both periods in between regularly scheduled scans in the 24-Hour Repeating scheduler, as well as in designated Cooling Times. To access the Scan on Demand scheduler, click on the vertical tab within the Schedule Upcoming Scans screen labeled Scan on Demand. However, all changes to the 24-Hour Repeating Schedule must either be applied to the IncuCyte™ by clicking the red Apply button, or reloaded to an unchanged state by clicking the Reload button before the Scan on Demand scheduler can be accessed. If changes are not applied or reloaded, the Scan on Demand tab will be unavailable.

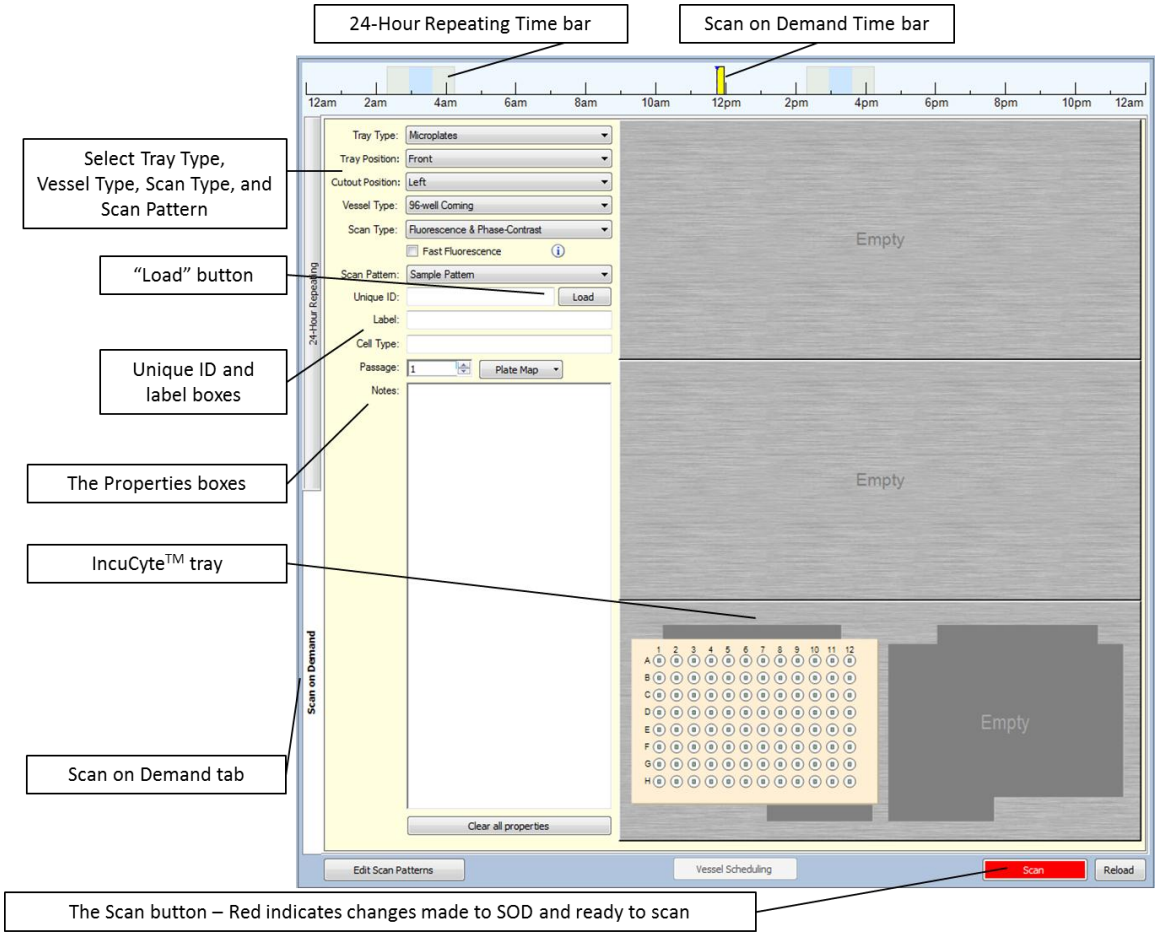


Figure 7-3. Scan on Demand Screen.

Within the Scan on Demand scheduler, the 24-Hour Repeating scan schedule (including all of the time bars) will be faded to the background, but will be clearly visible (See Figure 7-3). The current time will be indicated as in the 24-Hour Repeating scheduler, but will be followed by the yellow Scan on Demand Time bar. Similar to the 24-Hour Repeating schedule, the width of the time bar will

correlate to the amount of time required to scan the vessel inserted into the Scan on Demand Tray. Scan on Demand allows users to scan only one vessel in one tray position at a time. The Tray Type, Tray Position, Cutout Position, Vessel Type, and Scan Type must be entered into the scheduler prior to scanning.

In addition to a Label, users can also supply a Unique ID to each vessel. This Unique ID can either be generated manually by typing or pasting information into the Unique ID field, or a Unique ID correlating to the current year/day/time will be assigned to the Scan on Demand vessel. This information can be used to link subsequent scans together. However, it is important to note that if the user fails to type in, or select (using the load button) the correct Unique ID, scans will not be linked together, and cannot be accomplished post scanning.

Clearly, additional scheduling rules apply to the Scan on Demand scheduler in order to protect regularly scheduled scans from being skipped. These rules include:

- The Scan on Demand Time bar is typically yellow. If the Scan on Demand Time bar is Red, this means that the Scan on Demand scan time overlaps with a scan time in the 24-Hour Repeating Scheduler. Clicking the Red scan button will result in an error.

- The estimated time (indicated by the width of the yellow bar) to scan in the Scan on Demand mode is ≈20% longer than the estimated time in the 24-Hour Repeating scheduler. This additional time is required so that the user has time to replace the Scan on Demand vessel with the 24-Hour Repeating vessel.

Scanning using the Scan on Demand Scheduler is initiated by clicking the red Scan button (For additional information, please refer to Section 8.5.9, Page 83.

IMPORTANT:

1. Remember, the scanning process causes the IncuCyte™ to become warm. In order to prevent overheating, it is recommended that total scan time not exceed 45 minutes. Although the Scan on Demand function can be used during cooling times and between scan times, it is recommended that you do not scan too often.

2. Scans of vessels in the Scan on Demand mode cannot be linked together using the Unique ID following a scan. The Unique ID must be entered prior to scanning. Furthermore, scans of vessels in the Scan on Demand mode cannot be linked to vessels in the 24-Hour Repeating scheduler.

3. All changes to the 24-Hour Repeating schedule must be Applied or Reloaded in order to access the Scan on Demand mode.

4. Users MUST replace the 24-Hour Repeating vessels into the IncuCyte™ following a Scan on Demand. Failure to do so can result in several problems including failed/skipped scans, or insertions of incorrect, Scan on Demand vessel scans into the 24-Hour Repeating scheduled vessel scan sequence.

7.1.5 ImageLock™ Mode

Essen BioScience manufactures a special 24 and 96-well plate type called the Essen ImageLock™ plate. These plates facilitate very precise, repeated imaging within each well. Under standard imaging conditions, slight variations can occur in the location of imaging from one scan to the next, resulting in small jumps between frames. When very precise, repeated imaging is required – for instance, when generating a movie – use the Essen ImageLock™ plate. By selecting an ImageLock™ plate and running it in ImageLock™ mode, the highest fidelity movie will be generated.

If an ImageLock™ plate is selected as the vessel type (under the Physical Layout tab), the Scan Type box directly beneath will include a Phase-Contrast ImageLock™ option (Figure 7-4). While the default setting will be Phase-Contrast ImageLock™, an ImageLock™ plate can be run in either Phase-Contrast ImageLock™ or Phase-Contrast mode.

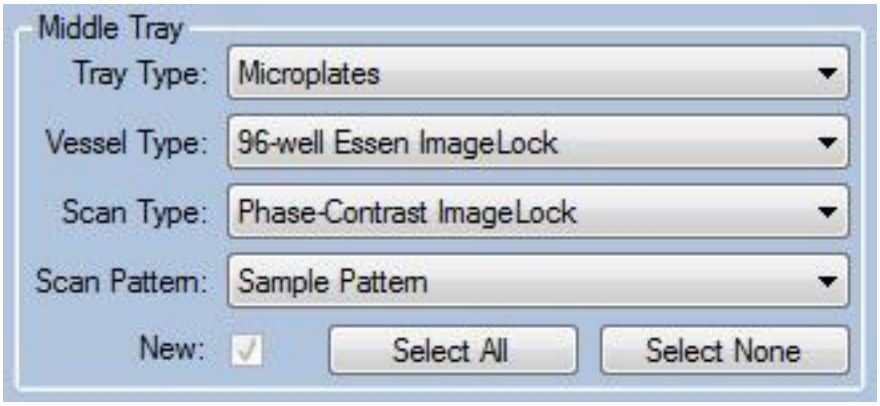


Figure 7-4. Running in ImageLock Mode

If an ImageLock™ plate is run in the standard Phase-Contrast mode, then locking will not occur, and the results will be comparable to those of a "standard" (non-ImageLock™) plate.

NOTE: It is recommended that ImageLock™ plates ONLY be run in Phase-Contrast ImageLock™ mode.

For information on obtaining the Essen ImageLock™ plate, contact Essen BioScience. Sales information is included at the end of this manual, Section 13 (page 244).

FLR

7.1.6 ImageLock™ Mode FLR

With an FLR instrument, simply select Fluorescence & Phase-Contrast ImageLock™ from the Scan Type pull-down menu displayed in Figure 7-4.

7.2 View Completed Scans

After scans have been generated, select the View Completed Scans task bar to view the results. See Figure 7-5.

The times and dates for all completed scans are displayed in the Time Tree. Select a specific time to display the scan for that time. The time and date of the scan currently displayed is shown at the top of the screen (02/26/08 @ 6:00am for Figure 7-5).

Highlight a specific vessel for a closer look by selecting the vessel with the mouse (left-click once). The selected vessel will be highlighted in yellow, and the details of the vessel will be displayed in the Selected Vessel box to the right of the trays. These details include:

- Image: All images obtained during the scan can be viewed under the Image tab.
- Properties: Selecting the Properties tab will display all the Properties entered when the scan was set including any Notes. Additional Comments can be added at this time.

- Metrics: Selecting the Metrics tab displays the quantitative results calculated from each image of each well or sector of the selected vessel. These values will be used to generate any graphical representations of the data.

7.2.1 Vessel View

A closer look at a specific vessel can also be generated by left double-clicking with the mouse on the selected vessel displayed within the tray. This will open a new window entitled Vessel View which displays the same details described above. Alternatively, select the Vessel View button at the bottom of the Selected Vessel box. See Section 8.7.3, page 131 for more information on the Vessel View window.

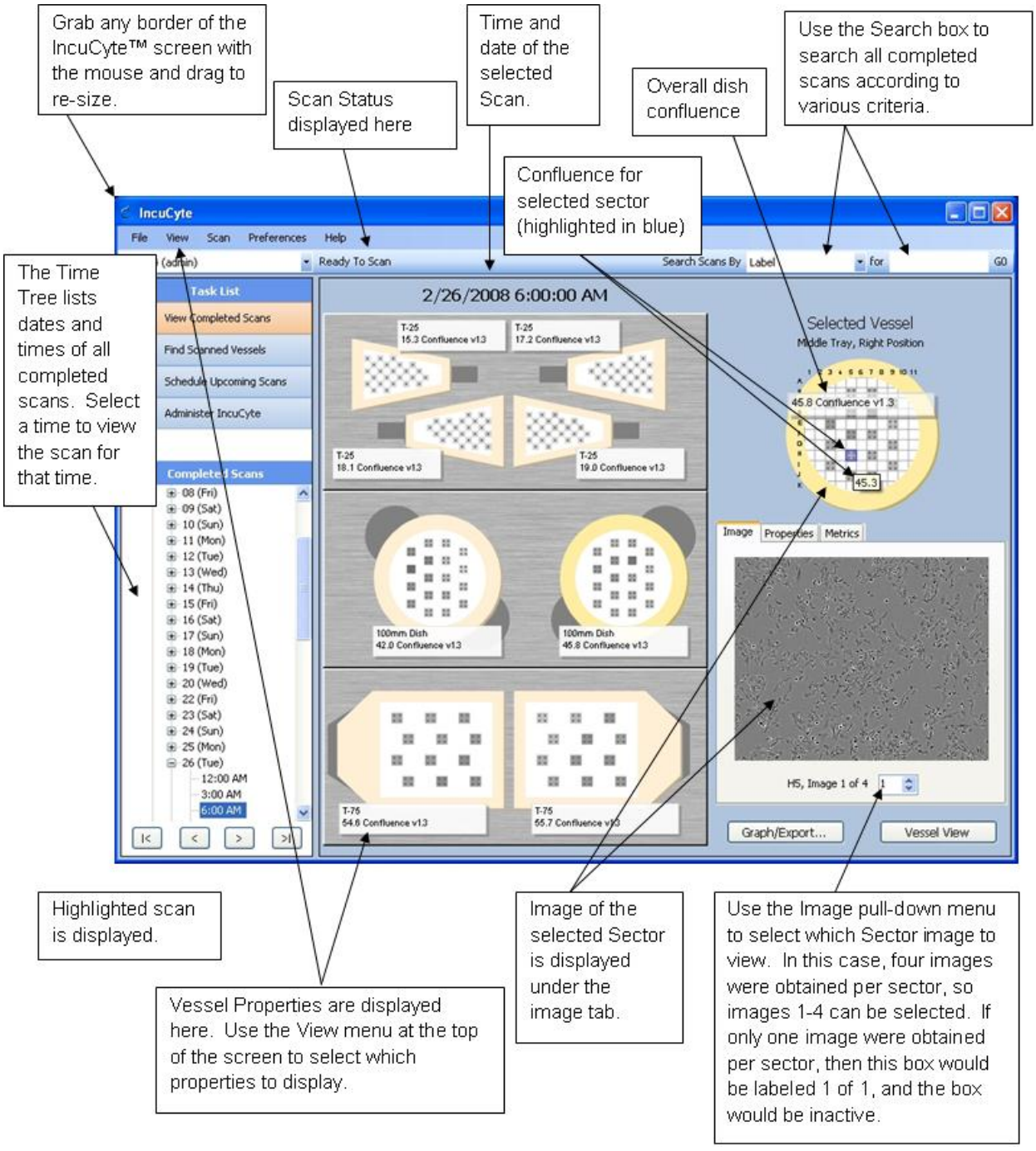

Grab any border of the IncuCyte™ screen with the mouse and drag to re-size.
Scan Status displayed here
Time and date of the selected Scan.
Overall dish confluence
Use the Search box to search all completed scans according to various criteria.
Confluence for selected sector (highlighted in blue)
The Time Tree lists dates and times of all completed scans. Select a time to view the scan for that time.
IncuCyte
File View Scan Preferences Help
Ready To Scan
Search Scans By Label for GO
Task List
View Completed Scans
Find Scanned Vessels
Schedule Upcoming Scans
Administer IncuCyte
Completed Scans
08 (Fri)
09 (Sat)
10 (Sun)
11 (Mon)
12 (Tue)
13 (Wed)
14 (Thu)
15 (Fri)
16 (Sat)
17 (Sun)
18 (Mon)
19 (Tue)
20 (Wed)
22 (Fri)
23 (Sat)
24 (Sun)
25 (Mon)
26 (Tue)
12:00 AM
3:00 AM
6:00 AM
2/26/2008 6:00:00 AM
T-25 15.3 Confluence v1.3
T-25 17.2 Confluence v1.3
T-25 18.1 Confluence v1.3
T-25 19.0 Confluence v1.3
100mm Dish 42.0 Confluence v1.3
100mm Dish 45.8 Confluence v1.3
T-75 54.6 Confluence v1.3
T-75 55.7 Confluence v1.3
Selected Vessel
Middle Tray, Right Position
45.8 Confluence v1.3
45.3
Image Properties Metrics
HS, Image 1 of 4 1
Graph/Export...
Vessel View
Highlighted scan is displayed.
Image of the selected Sector is displayed under the image tab.
Use the Image pull-down menu to select which Sector image to view. In this case, four images were obtained per sector, so images 1-4 can be selected. If only one image were obtained per sector, then this box would be labeled 1 of 1, and the box would be inactive.
Vessel Properties are displayed here. Use the View menu at the top of the screen to select which properties to display.

Figure 7-5. The View Completed Scans Screen

EX

7.2.2 View Completed Scans EX

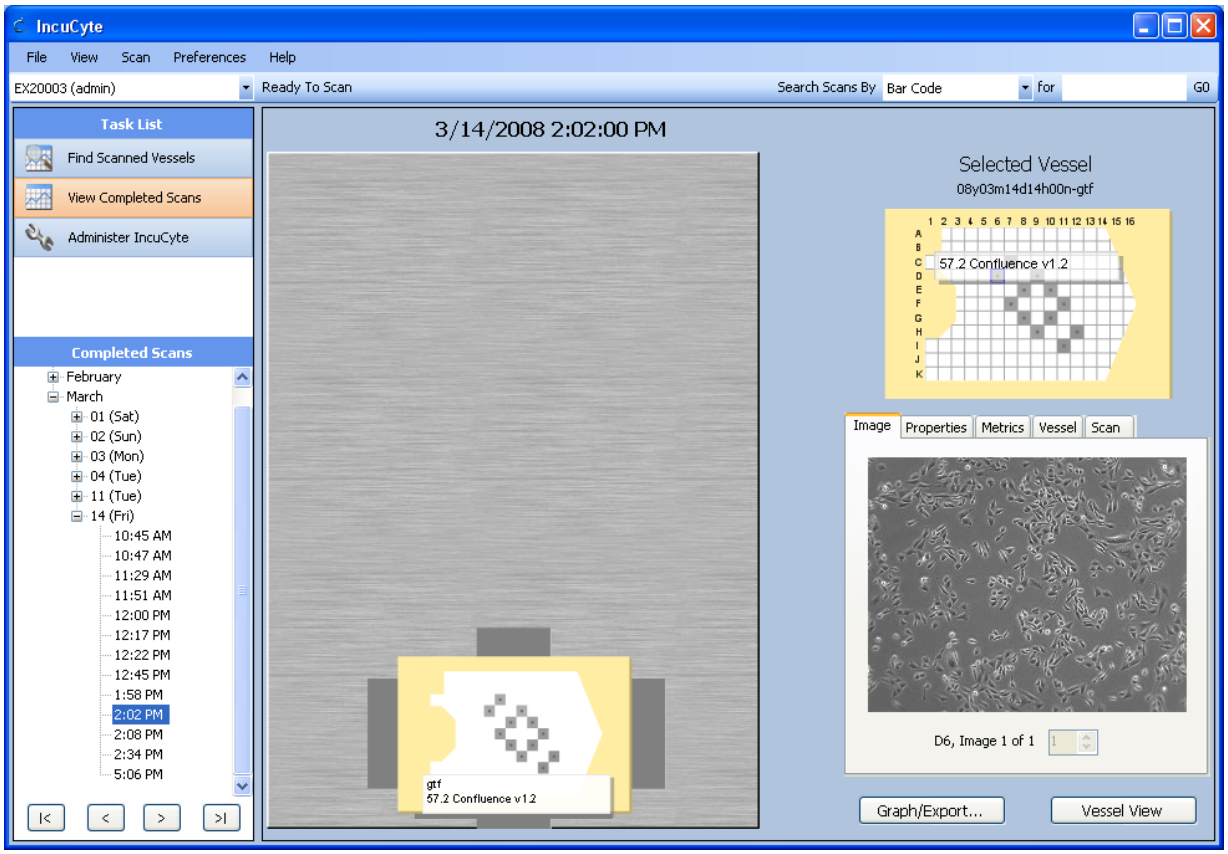


Figure 7-6. View Completed Scans Screen in the IncuCyte™ EX

When the Standard Model IncuCyte™ software is launched, it opens by default to the View Completed Scans screen; however, when the IncuCyte™ EX software is launched, it opens by default to the Find Scanned Vessels screen. This difference reflects the more "vessel-centric" nature of the IncuCyte™ EX. Nonetheless, the View Completed Scan screen can be selected in the EX by choosing the second task bar from the top. See Figure 7-6.

Note that in the EX, only a single tray is visible, and the Schedule Upcoming Scans task bar is absent. This is because scans in the EX must be scheduled through the automated cell handling system.

The Selected Vessel Box contains two extra tabs: Vessel and Scan (in addition to Image, Properties and Metrics). Also, the vessel barcode is displayed directly below the image of the Vessel. In Figure 7-7 the vessel barcode is CCF7234523HZ.

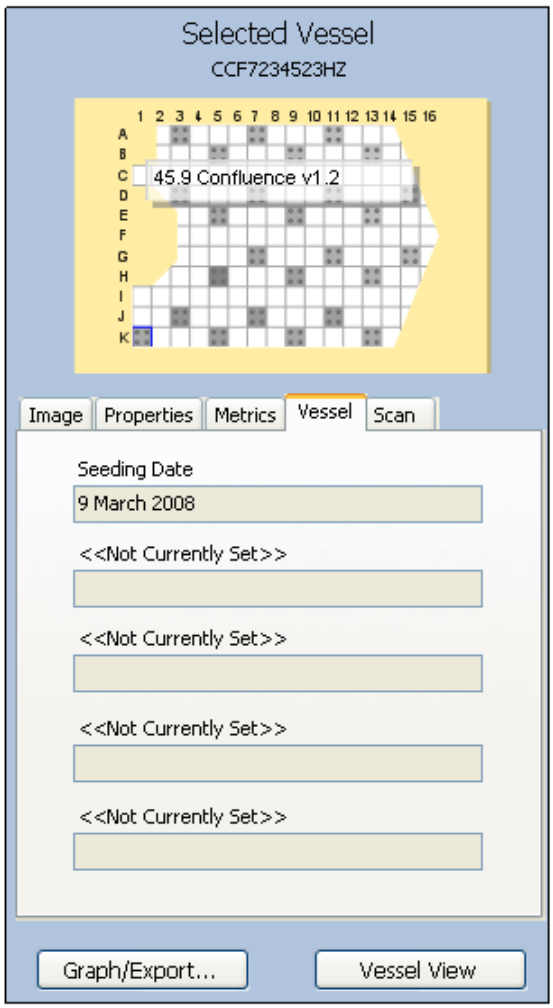


Figure 7-7. The Selected Vessel Box in EX

The properties within the Vessel and Scan tabs can be viewed locally, but they can only be populated remotely by the automated cell handling device. Other features of the View Completed Scans Screen are unchanged except that you can search vessels by Barcode. For more information on this subject, see Section 8.9, page 164.

7.3 Find Scanned Vessels

Select the Find Scanned Vessels task bar to open the corresponding screen. Use this screen to search for previously scanned vessels.

Note that in the Find Scanned Vessels screen, the Time Tree ONLY displays scan times for the selected vessel. In contrast, the View Completed Scans screen displays ALL scans that have occurred on the instrument.

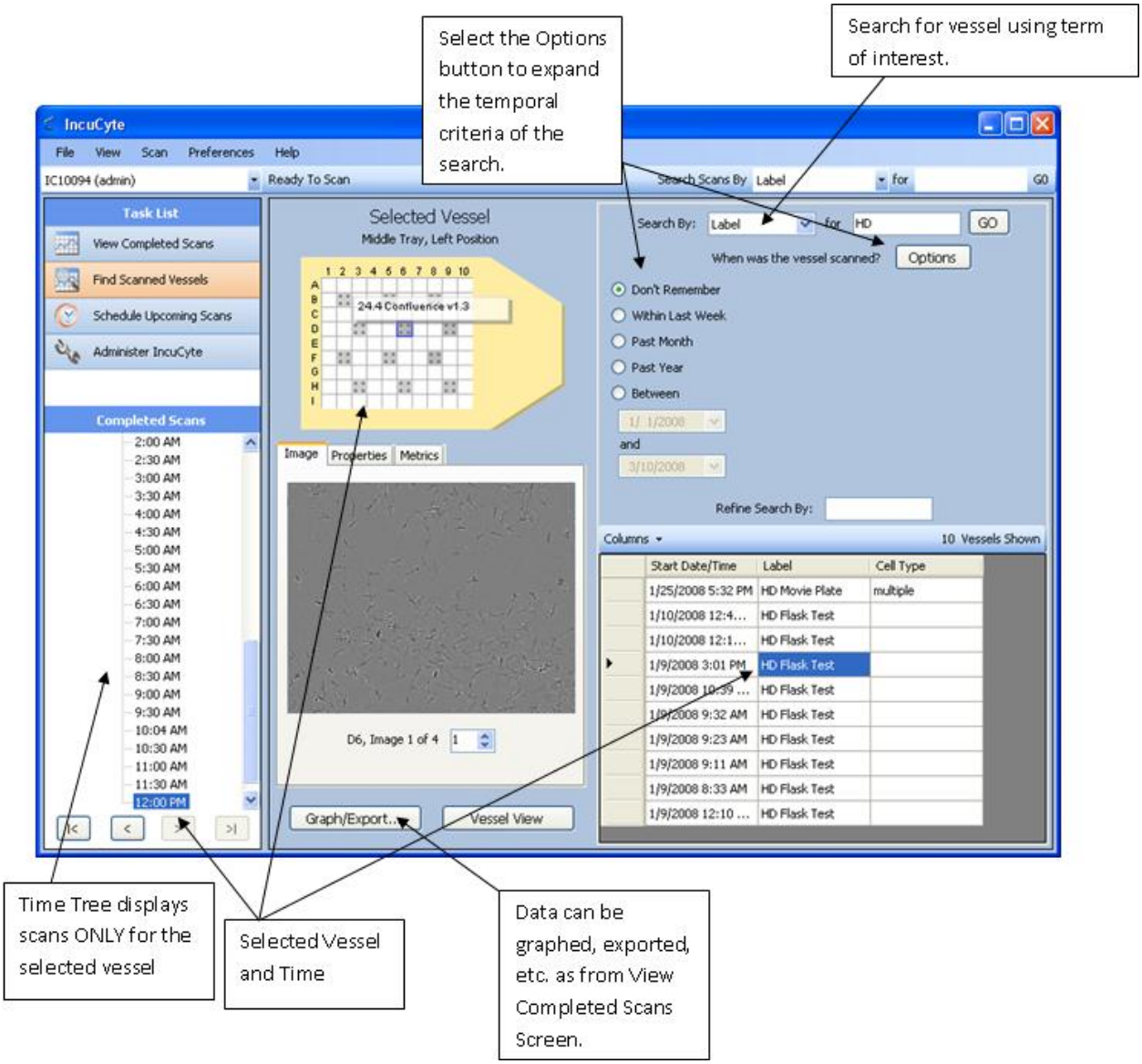


Figure 7-8. Find Scanned Vessels Screen

EX

7.3.1 Find Scanned Vessels EX

As mentioned in View Completed Scans, the Find Scanned Vessels Screen is the default screen that opens when the EX software is launched, and it is the jumping-off point for viewing scan results. Users of the Standard Model IncuCyte™ and the IncuCyte™ HD frequently set scan schedules involving a small number of vessels that are scanned many times. In contrast, the IncuCyte™ EX is specifically designed to scan an extraordinarily large number of vessels, each of which is scanned infrequently. Therefore, it is generally easier for the User to locate their particular vessel of interest via the Find Scanned Vessels screen. See Figure 7-9. For more information see Section 8.9, page 164.

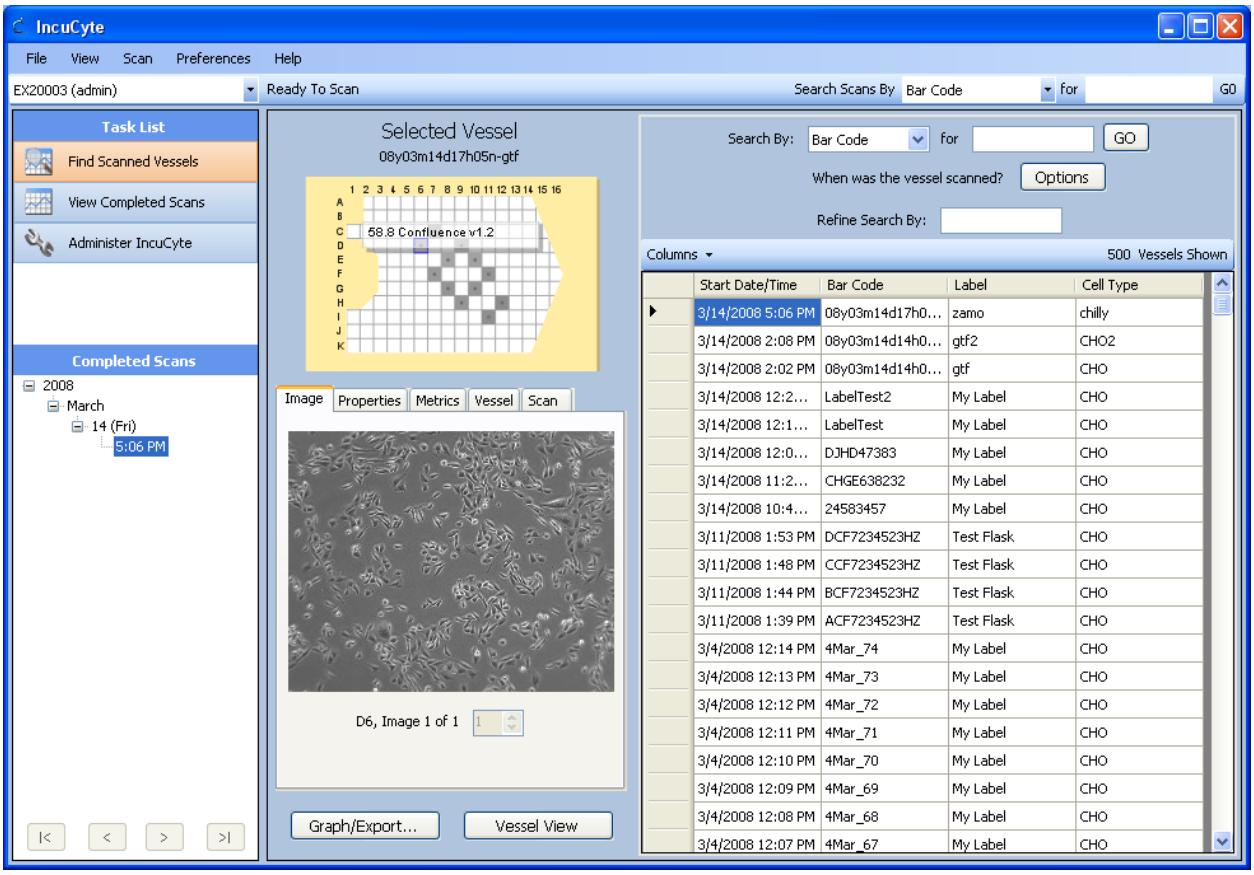

IncuCyte
File View Scan Preferences Help
EX20003 (admin)
Ready To Scan
Search Scans By Bar Code for GO
Task List
Find Scanned Vessels
View Completed Scans
Administer IncuCyte
Completed Scans
2008
March
14 (Fri)
5:06 PM
Selected Vessel
08y03m14d17h05n-gtf
58.8 Confluence v1.2
Image Properties Metrics Vessel Scan
D6, Image 1 of 1
Graph/Export...
Vessel View
Search By: Bar Code for GO
When was the vessel scanned? Options
Refine Search By:
Columns
500 Vessels Shown
Start Date/Time Bar Code Label Cell Type
3/14/2008 5:06 PM 08y03m14d17h0... zamo chilly
3/14/2008 2:08 PM 08y03m14d14h0... gtf2 CHO2
3/14/2008 2:02 PM 08y03m14d14h0... gtf CHO
3/14/2008 12:2... LabelTest2 My Label CHO
3/14/2008 12:1... LabelTest My Label CHO
3/14/2008 12:0... DJHD47383 My Label CHO
3/14/2008 11:2... CHGE638232 My Label CHO
3/14/2008 10:4... 24583457 My Label CHO
3/11/2008 1:53 PM DCF7234523HZ Test Flask CHO
3/11/2008 1:48 PM CCF7234523HZ Test Flask CHO
3/11/2008 1:44 PM BCF7234523HZ Test Flask CHO
3/11/2008 1:39 PM ACF7234523HZ Test Flask CHO
3/4/2008 12:14 PM 4Mar_74 My Label CHO
3/4/2008 12:13 PM 4Mar_73 My Label CHO
3/4/2008 12:12 PM 4Mar_72 My Label CHO
3/4/2008 12:11 PM 4Mar_71 My Label CHO
3/4/2008 12:10 PM 4Mar_70 My Label CHO
3/4/2008 12:09 PM 4Mar_69 My Label CHO
3/4/2008 12:08 PM 4Mar_68 My Label CHO
3/4/2008 12:07 PM 4Mar_67 My Label CHO

Figure 7-9. The Find Completed Vessels Screen for the IncuCyte™ EX

7.4 Graphing

7.4.1 Create a Graph

After scan data have been collected, they can be used to generate graphs of the selected metric over time. The IncuCyte™ graphing feature can be accessed in one of 4 ways:

- By selecting the Graph/Export button in the Selected Vessel box in the Completed Scans screen. This will open the Graphing Box.
- By selecting the Graph tab in the Vessel View window (Section 8.7.3, page 130).
- Choosing the Graph option from the Scan pull-down menu (Section 8.7.9, page 152).
- Right-click on a vessel in the View Completed Scans screen (See Tasks Available by Right-Clicking on a Selected Vessel, page 131)

The Graphing Box will now appear. See Figure 7-10 Follow the instructions to create a graph. The newly created graph can be subsequently customized according to desire. See Figure 7-11.

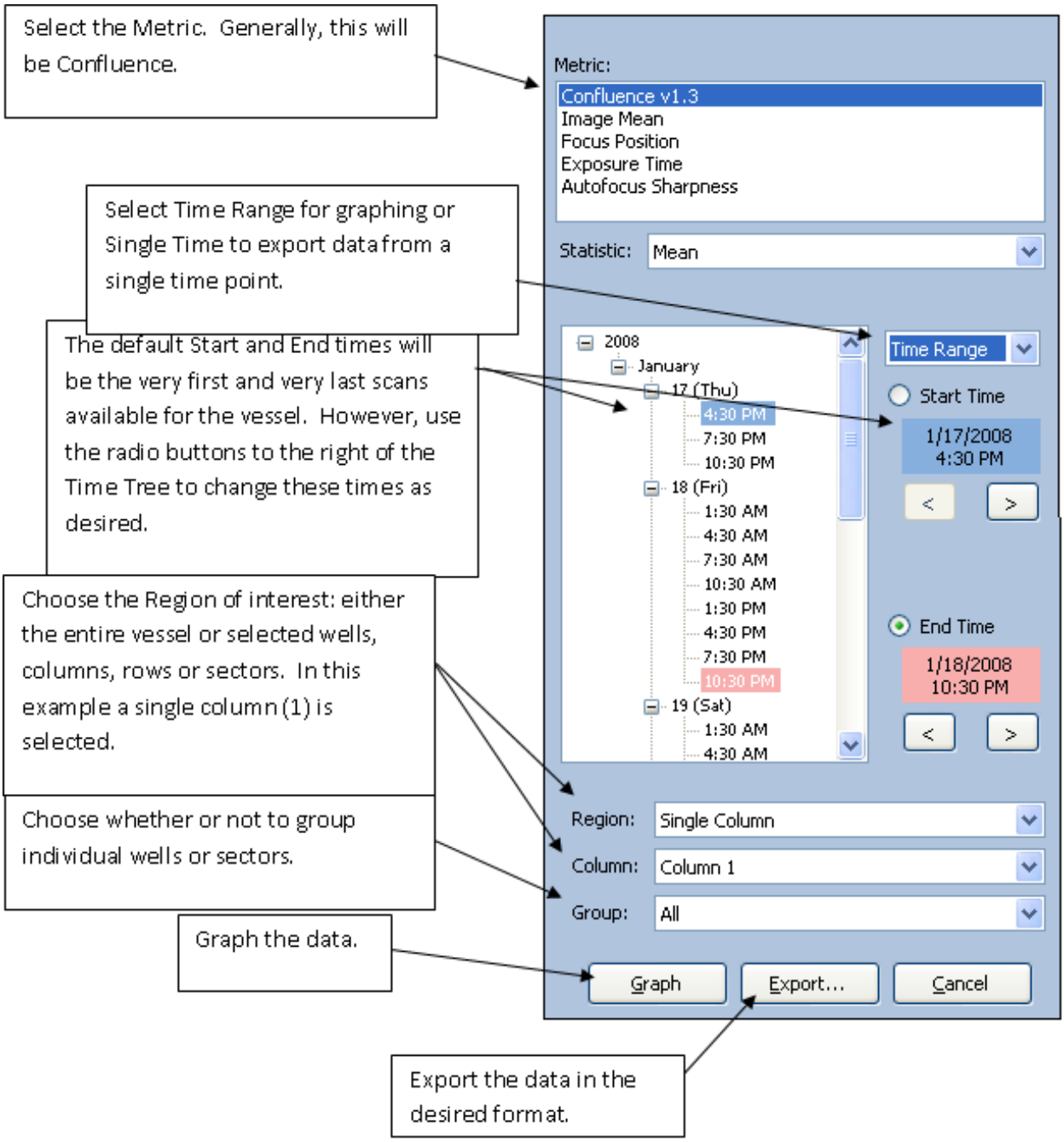


Figure 7-10. The Graphing Box

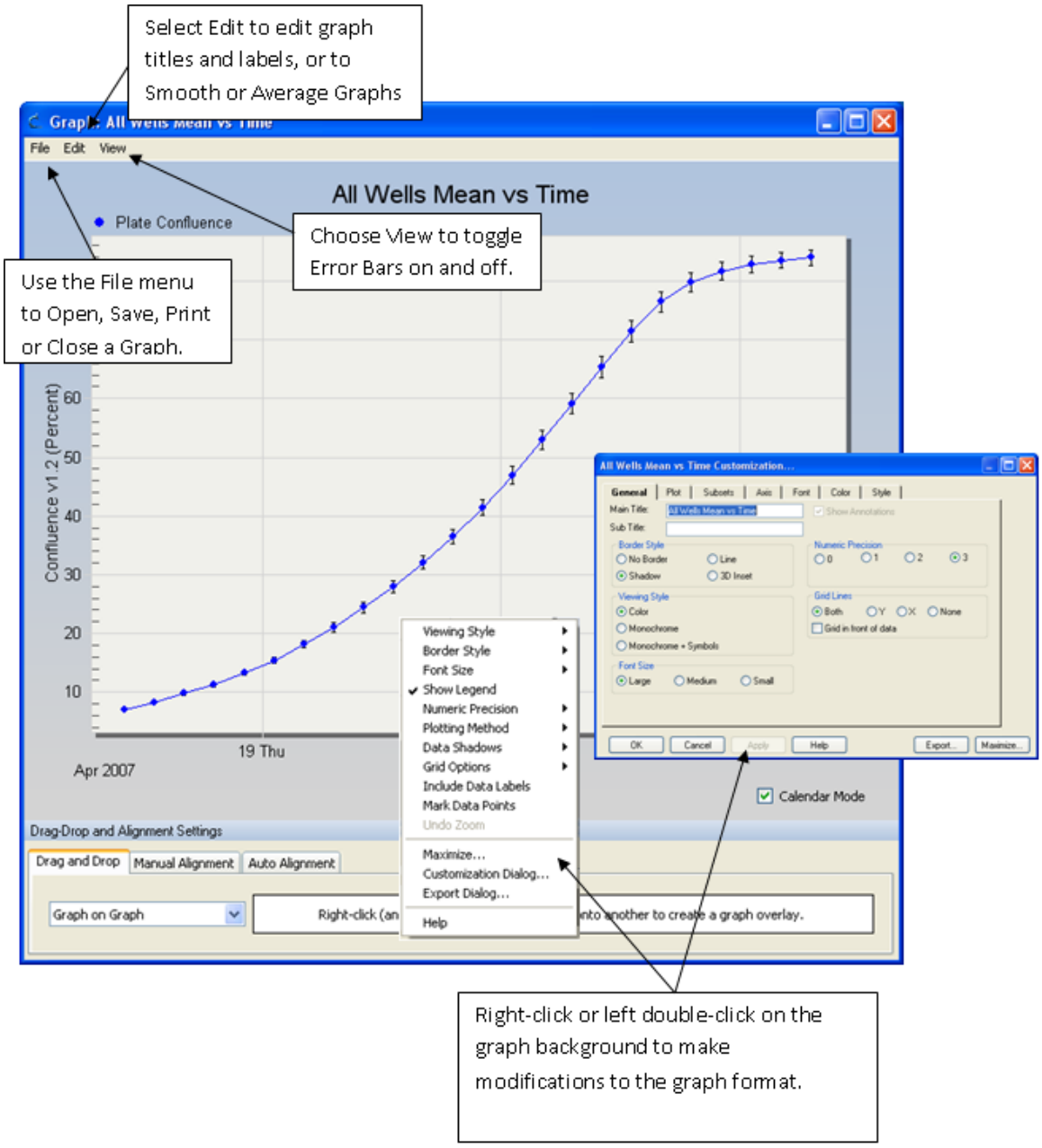


Figure 7-11. Graph Example

Note that the Graphing Box looks somewhat different when accessed via the Vessel View as opposed to the View Completed Scans screen. In the Vessel View, the Graphing Box contains a space for a Histogram and an option for Microplate Graphing. Currently, histograms are only available with the IncuCyte™ FLR Object Counting Application and Microplate Graphing is only available when Graph/Export is selected via the Vessel View Menu under Utilities or through the Graph/Export tab in the Vessel View screen.

Dragging Graphs

Graphs can be dragged to either:

- Create a Plot Overlay (within the IncuCyte™ software) or
- Drag the entire plot (image or raw data) into a different software program such as EXCEL®, MS Word®, PowerPoint® or Outlook®.

To Drag and Drop a graph, choose the Drag and Drop tab at the bottom of the graph window. Then use the pull-down menu to select the appropriate Drag and Drop option:

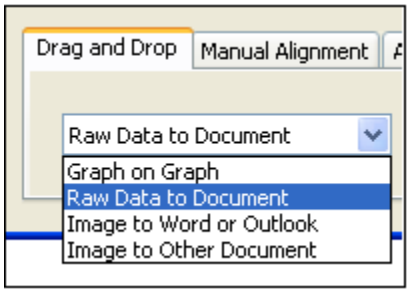


Figure 7-12. The Drag and Drop Pull-Down Menu

- Plot Overlay: For example, in a multiwell plate, graph two wells individually. Then, right-click on the graph for the first well and drag it onto the plot for the second well. Now, both traces will appear on a single plot. Use the Delete button under the Manual Alignment tab to delete a trace that has been dragged but is subsequently not desired. See Figure 7-13. To create a Plot Overlay the Graph on Graph option must be selected.

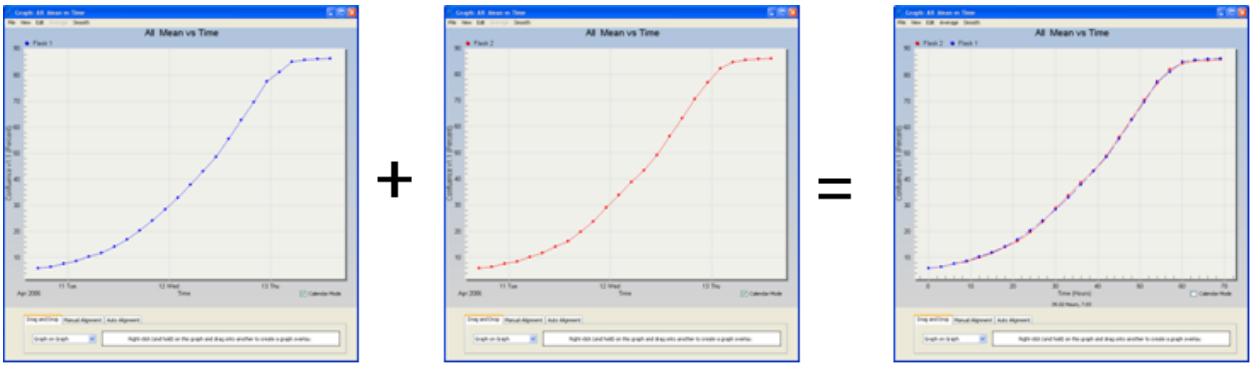


Figure 7-13. Use the mouse to drag the trace from one graph to the next to create a plot overlay.

- Alternatively, plots can be dragged into a different software program. The data can be dragged either in the form of the completed plot or in the form of raw data. Sometimes, for example, it might be convenient to copy the raw data into EXCEL®. To do this, simply right-click on the plot to be dragged (be sure that Drag and Drop: Raw Data to Document). The raw data will be copied into the new spreadsheet and can be manipulated within the EXCEL® software. See Figure 7-14.

The raw data can also be exported into EXCEL® using the Export button See Section 8.10.3, page 172 for more information on exporting raw data.

Date Time	Elapsed	Column 1	Column 2
4/5/2006 15:45	0	5.116157	5.62277
4/5/2006 18:45	3	5.873641	6.483157
4/5/2006 21:45	6	6.748467	7.517222
4/6/2006 0:45	9	7.93768	8.310006
4/6/2006 3:45	12	9.286036	9.822143
4/6/2006 6:45	15	10.79348	11.6282
4/6/2006 9:45	18	12.91238	13.6381
4/6/2006 12:45	21	15.41242	15.66134
4/6/2006 15:45	24	18.40523	19.5973
4/6/2006 18:45	27	22.04792	23.65909
4/6/2006 21:45	30	26.11257	28.86244
4/7/2006 0:45	33	30.45502	34.0718
4/7/2006 3:45	36	35.27699	39.10634
4/7/2006 6:45	39	40.49081	44.93753
4/7/2006 9:45	42	46.00566	50.74551
4/7/2006 12:45	45	51.61444	56.49158
4/7/2006 18:45	51	63.54694	69.3348

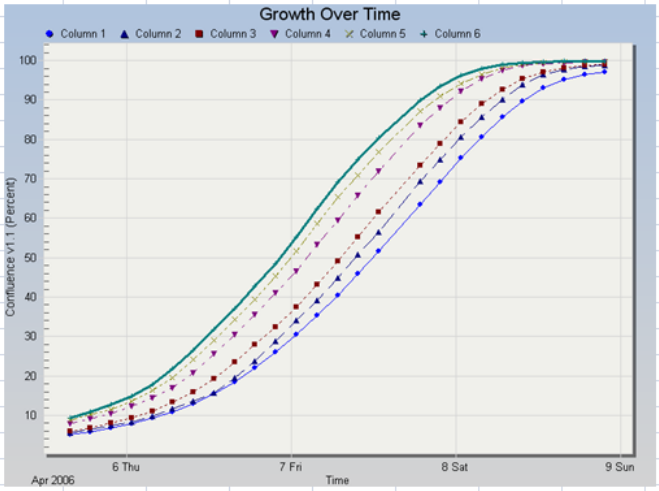


Figure 7-14. Graph dragged into EXCEL® as Raw Data (left) and as Image (right).

Summary:

To drag one plot onto another (within the IncuCyte™ graphing software) select Drag and Drop as Graph on Graph.

2. To drag the graph image into MS Word® or MS Outlook®, select Drag and Drop as Image to Word or Outlook.

3. To drag and drop the image into other software programs, select Drag and Drop as Image to Other Document.

4. To drag and drop other raw data, select Drag and Drop Raw Data to Document.

5. After a Plot Overlay has been created, all dragged traces can be subsequently deleted using the Delete button (under the Manual Alignment tab). However, the original trace(s) for the plot that the other traces were dragged onto CANNOT be deleted.

7.4.2 Creating Average Graphs

When multiple traces are included on a single plot (either as a result of dragging or by graphing all wells/sectors), these traces can be combined to create a single, Average Graph. To do this, select the Edit pull-down menu from the top of the Graph window, and then select Average Lines Together. Select OK in the dialog box that appears, and a new, Average graph will be created in a new window. Note that the Average Graph function does not generate a graph with

error bars. See Section 8.10.5, Error Bars (page 187) for information on error bars within a single vessel.

The Average graph function can be also used to combine graphs from different vessels and/or from different days.

NOTE: Error bars are not available on graphs created using the Average Function. To create a graph with error bars, see Section 8.10.5, page 187.

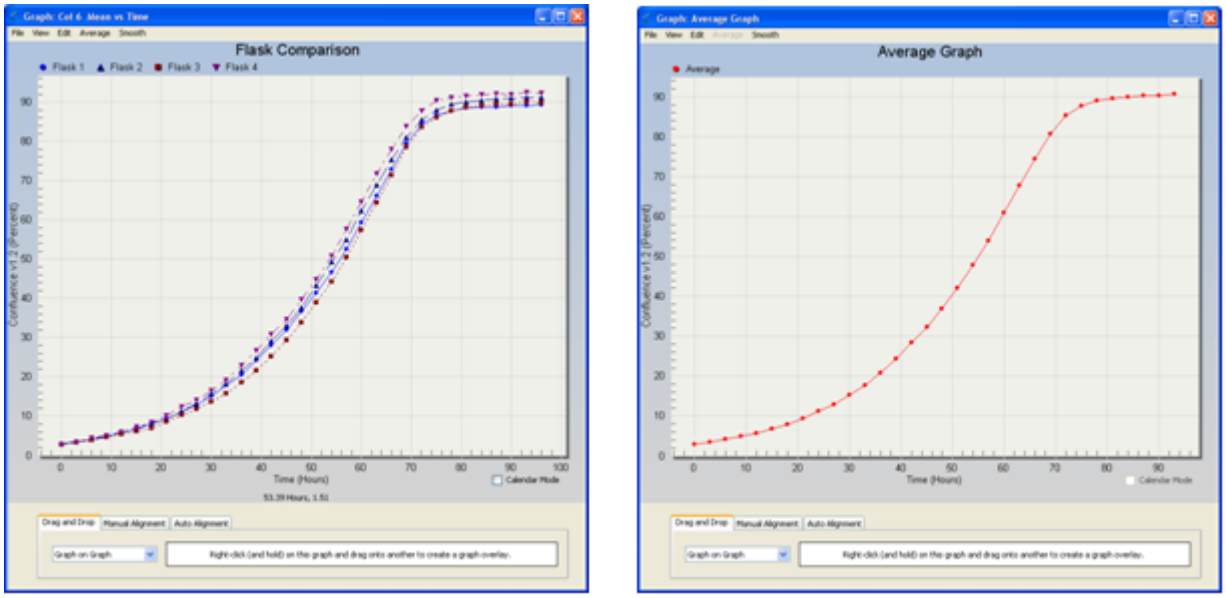


Figure 7-15. Creating an average graph

7.4.3 Export of Graph Data

Data can be exported by selecting the Export button underneath the graph settings. Export parameters can be adjusted according to the desired Layout, Destination and Other options. Further reading on this function is recommended. See Section 8.10.4, Export Metrics, page 172.

When generating the selected Metrics, the stringency of outlier removal can be adjusted by selecting the Preferences option at the top of the screen. Select from None (no outlier removal), Standard or Aggressive. Whenever a graph is created, it will conform to the currently selected degree of stringency. None is the IncuCyte™ default setting, and it is recommended. See Section 8.10.5 (Preferences: Outlier Removal and Drag and Drop Auto Alignment, page 184) for additional information on outlier removal. Outlier removal applies to all Metrics.

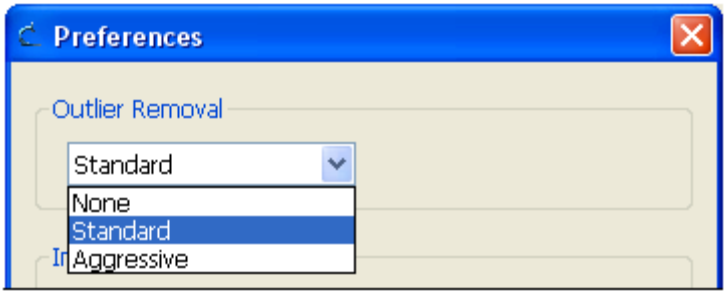


Figure 7-16. The Preferences Window

The Preferences window contains some additional options that relate to dragging and dropping graphs and images. See Sections: 8.10.5, Preferences: Outlier Removal and Drag and Drop Auto Alignment (page 184) for more information on these options.

Note that increasing the stringency of outlier removal does not necessarily result in a smoother graph. To reduce noise in the graph, see Section 8.10.5 regarding the Smooth feature.

7.4.4 Saving and Printing Graphs

Graphs can be saved or printed using the File pull-down menu at the top of the plot. If a graph has been created and saved within the IncuCyte™ software, it can ONLY be opened again through the IncuCyte™ software (using this menu selection). If the graph file is selected by another method (for example, just locating the file on the hard drive and clicking on it), it will not open. If, however, a graph is created and then saved into another software program (for example, MS Word®), it can be opened directly through that program. In this case, however, it will be a static image that cannot be manipulated.

8 Software Reference

The software automatically opens to the View Completed Scans window (under the Task List bar). The first time the software is opened, the screen will be empty (Figure 8-1). However, once scans have been performed, the software will open to show the very last scan that was completed.

EX

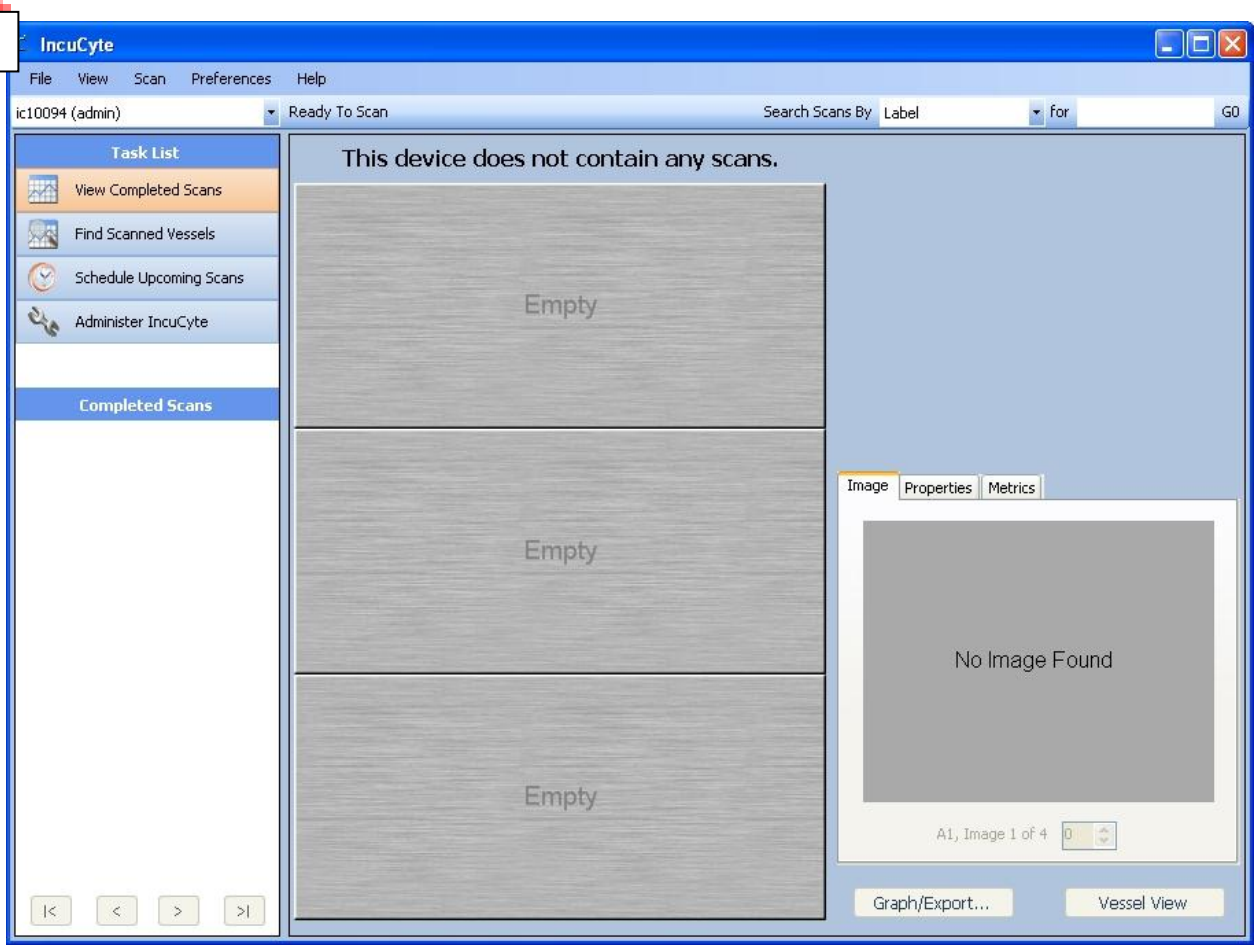


Figure 8-1. View Completed Scans Screen with no scans completed

Note that the IncuCyte™ EX automatically opens to the Find Scanned Vessels screen. The View Completed Scans screen is still accessible in the EX, but it is not the default setting.

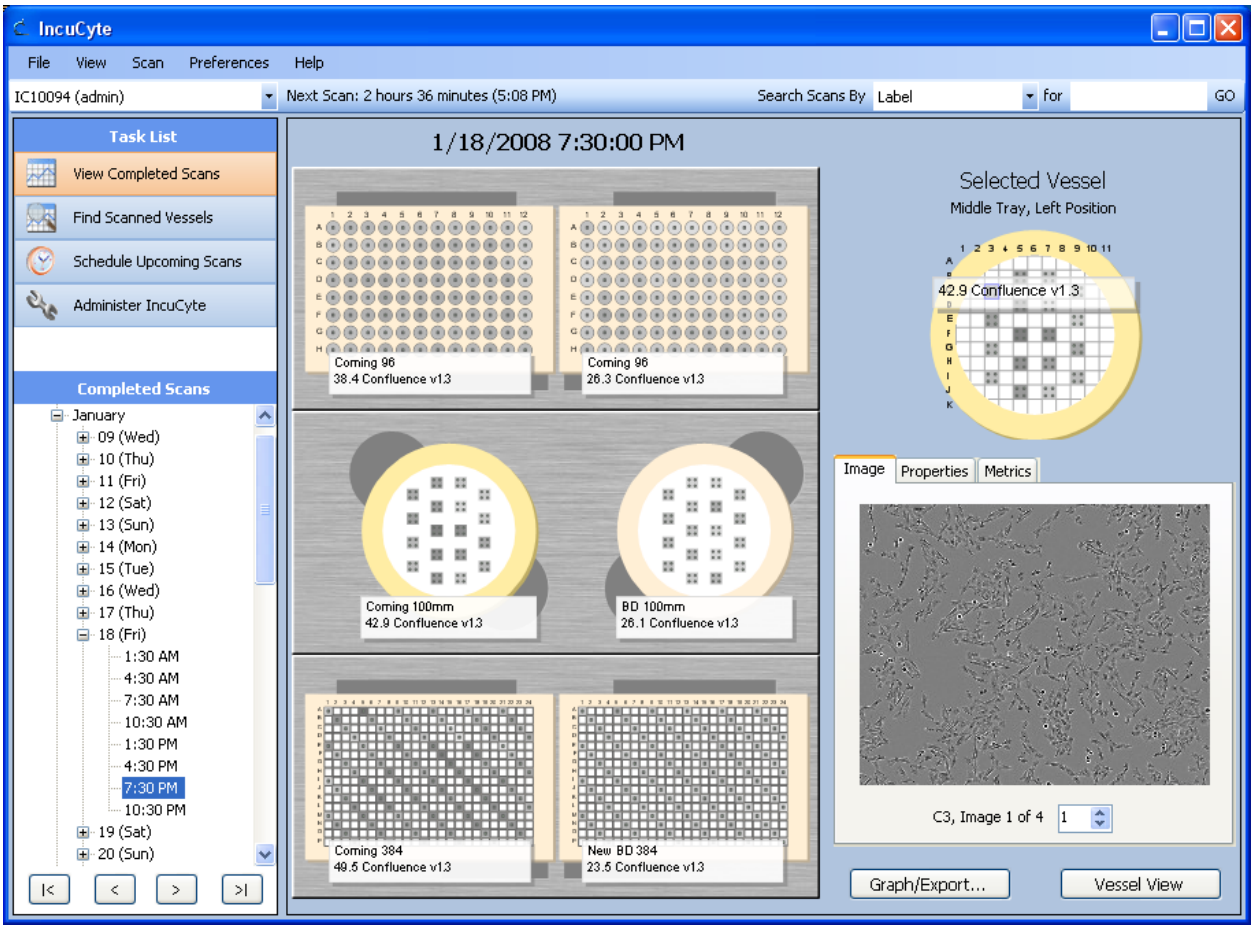


Figure 8-2. View Completed Scans Screen with scans completed

8.1 Connections Box

The Connections box is located at the top, left corner of Figure 8-2. Also see Figure 8-3. It shows the instrument name (ICXXXXX) and the User name, or the name of the archive. If the User is connected to only one instrument or archive file, then selecting the Connections Box pull-down menu will not reveal any additional options. However, it is possible to be logged onto multiple instruments/archives simultaneously. In Figure 8-3, the User (MeMe) is logged into one instrument (IC10094) and two archive files (X:\...). It is possible to toggle between these multiple connections by using the Connections Box pull-down menu.

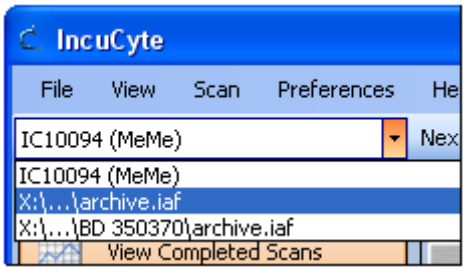


Figure 8-3. The Connections Box. Three connections are open: one instrument (IC10094) and two archives.

8.2 Menu Bar

File

Use the File Menu to open and close a connection to an instrument or archive (Section 8.1), open a previously saved graph, and exit the software.

View

Use the View menu to select what information is displayed for each vessel on the screen. See Figure 8-81. Select the characteristics displayed under the View Completed Scans Screen.

Scan

Use the Scan menu to search through scans and perform specific actions on a selected vessel, including browsing between scans, Vessel View and Graph, and Archive or Delete Vessel.

FLR

View Analysis Jobs brings up a window displaying all analysis jobs performed on an IncuCyte™ FLR. Double-click to open a desired job (See Section 9.3: FLR-Specific Software Applications)

Preferences

The Preferences menu contains options for viewing/exporting data and setting the speed of the Slideshow option in Vessel View (Section 8.7.6, Slideshow Mode).

Help

The Help menu contains links to the User Manual, IncuCyte™ hardware and software specifications, and a list of unavailable vessel trays (Section 8.5.1).

Note: To easily send instrument specifications to Essen BioScience for technical support, select About and then Copy To Clipboard to create a text file of IncuCyte™ specifications that can be emailed directly to Essen. For additional support information, refer to Section 11.2, page 241.

8.3 Next Scan Time

To the right of the Connections box, the Device Status is indicated. This typically lists the next time a scan will be initiated. If scanning is active, it will list the progress of the scan. If a device test is in progress, the status is indicated here.

EX

The Next Scan Time will not typically be displayed on the IncuCyte™ EX because the next scan time is determined by the automated cell handler. However, it is important to remember that even though no Next Scan Time is displayed, it does NOT mean that no scans are scheduled to occur.

8.4 Task Bars

The IncuCyte™ Task Bars allow the User to select the main screen display, or User interface. There are four interface options. Each of these options will be further discussed on an individual basis.

View Completed Scans

This is the screen currently visible in Figure 8-4. It shows the time and date of the selected scan in bold at the top and the results of the scan below.

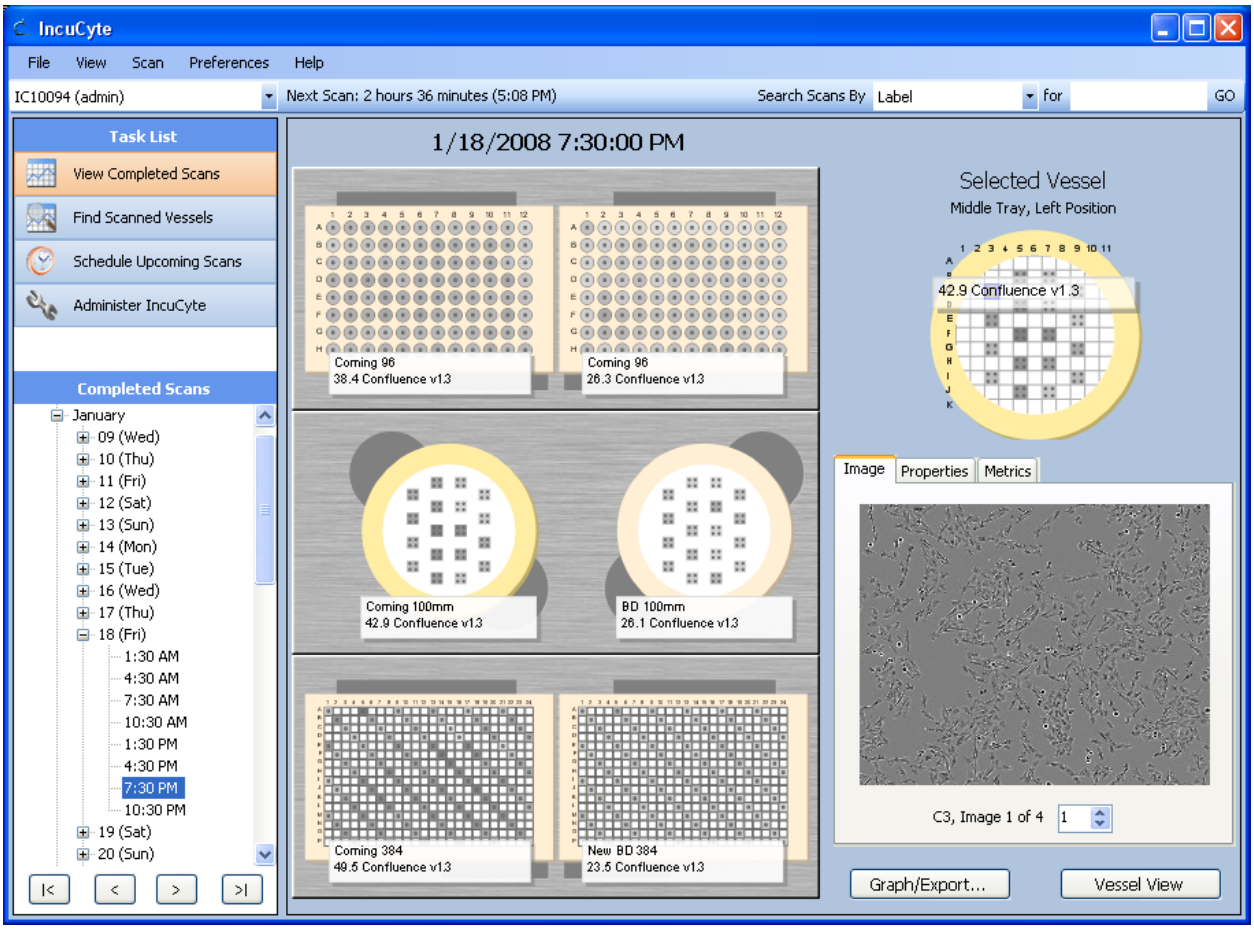


Figure 8-4. View Completed Scans Screen

Find Scanned Vessels

Search for individual scanned vessels.

Schedule Upcoming Scans

Use this screen to schedule scans.

EX

Note that the Schedule Upcoming Scans Task Bar is NOT available for the IncuCyte™ EX.

Administer IncuCyte™

This screen facilitates access to the administrative features of the IncuCyte™, including instrument logs, calibration and changing the password and User/account management.

8.5 Scheduling Scans

EX

Many of the functions covered in this Section will not be relevant to the IncuCyte™ EX User. The two functions that ARE relevant regard setting Scan Patterns and setting vessel Properties. Each of these sections is discussed for general IncuCyte™ use, followed directly by an additional section related to the specifics as they pertain to the EX. We strongly recommend that Users read both the general information regarding Scan Patterns and vessel Properties and then read the special sections specific to the EX. See the following sections:

Section 8.5.3: Setting Scan Patterns, page 67

Section 8.5.4: Setting Scan Patterns EX, page 70

Section 8.5.5: Setting Vessel Properties, page 73

The microscope can heat up during excessive scanning. Therefore, we recommend keeping scan times under 45 minutes and duty cycles under 50%. As an example, 40 minute long scans scheduled every three hours results in a duty cycle of 22.2% (40 minutes/180 minutes). Under these conditions, any local temperature rises will typically be less than 1 degree C and relatively short-lived.

The IncuCyte™ is capable of collecting nearly 1500 images in 40 minutes, so another good reason to avoid such “over-scanning” is to limit disk usage and processing time. This will keep hard drives from filling up unnecessarily and keep the system more responsive. We have found that between 0.5 and 1 image per square centimeter is usually sufficient to characterize the growth of cells in most tissue culture vessels. For example 48 images will usually characterize a T75 flask very well. With good technique that results in uniform seeding, even fewer images can be used. Also, with some slower growing cell types scanning every 4 or even 6 hours may be sufficient.

After connecting to your IncuCyte™, press the Schedule Upcoming Scans task bar. The first time this screen is opened, it will be empty (Figure 8-5).

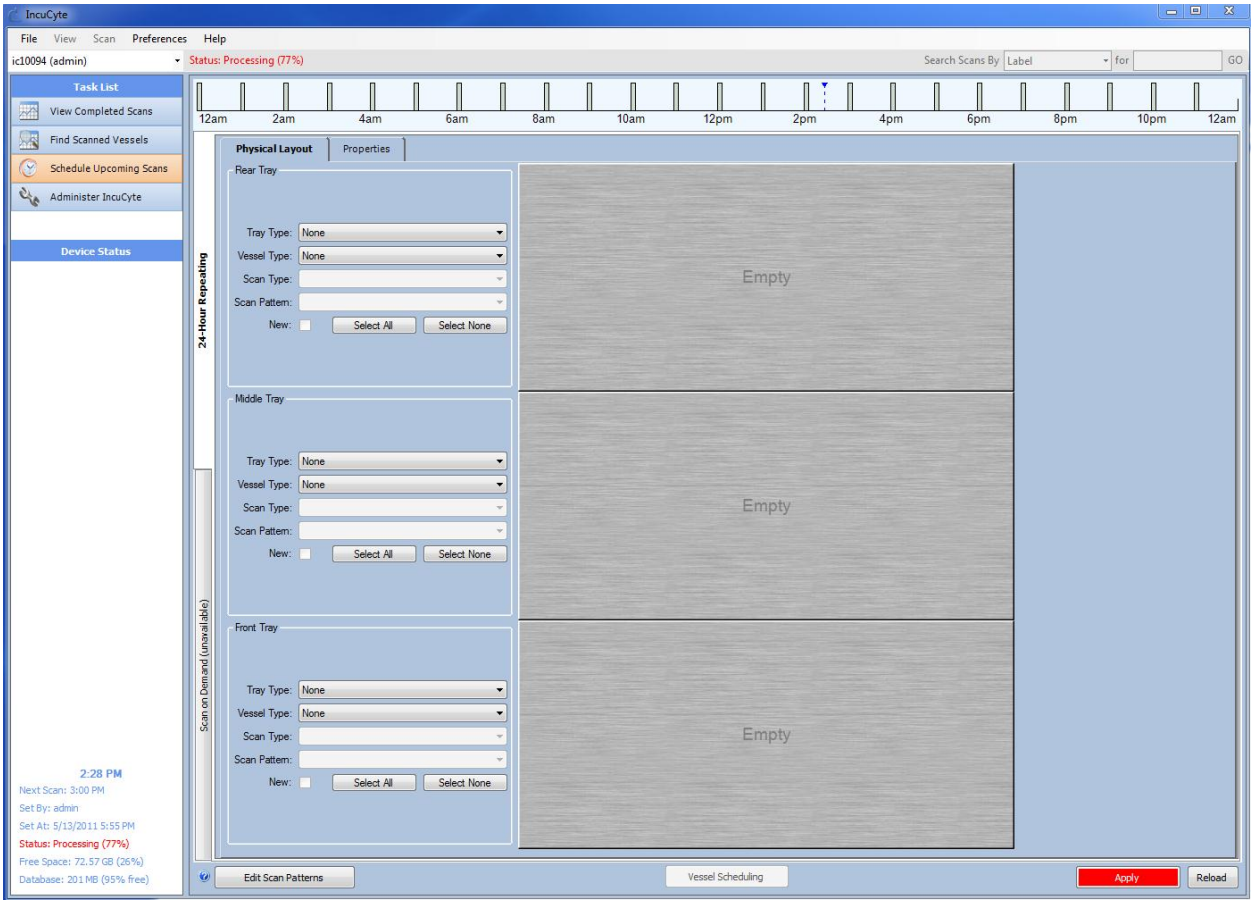


Figure 8-5. The empty Schedule Upcoming Scans screen

8.5.1 Physical Layout

Tray Type

Use the pull-down menu to select a Tray Type. The Tray Types are interchangeable, and a maximum of 3 trays fit into the drawer at any given time. Trays do not have to be present in all available spaces for the IncuCyte™ to function. There are different Tray Types to accommodate the different available flasks and dishes; however, a single Tray Type accommodates all available microplates. If no tray will be used, select None. All 3 trays must be individually selected. If unsure which Tray Type to use, select Find from the Tray Type pull-down menu.

Find

Selecting Find will bring up a new window listing all compatible flasks, dishes and microplates along with their appropriate Tray Types. Double clicking on a highlighted row will open the corresponding tray in the IncuCyte™ screen. The contents of the Find window are organized under separate tabs for Flasks, Plates, Dishes and Slides. The vessels within each tab are further organized under several headings.

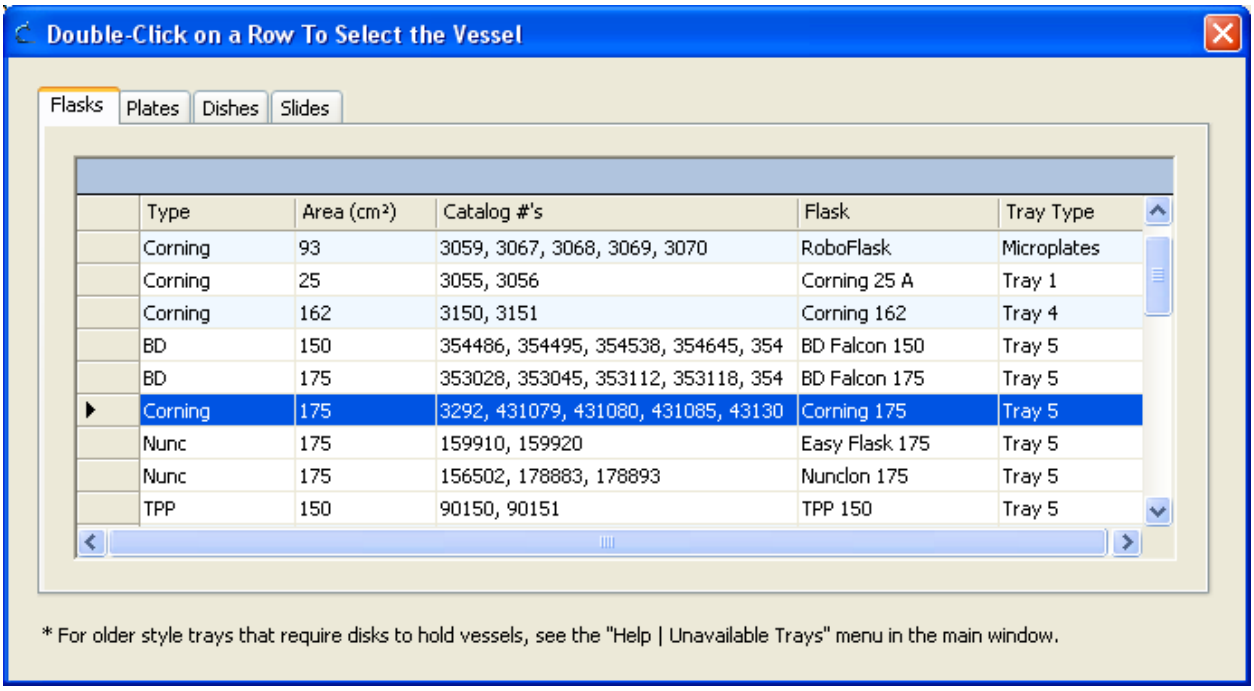

Double-Click on a Row To Select the Vessel
Flasks
Plates
Dishes
Slides
Type
Area (cm²)
Catalog #'s
Flask
Tray Type
Corning 93 3059, 3067, 3068, 3069, 3070 RoboFlask Microplates
Corning 25 3055, 3056 Corning 25 A Tray 1
Corning 162 3150, 3151 Corning 162 Tray 4
BD 150 354486, 354495, 354538, 354645, 354 BD Falcon 150 Tray 5
BD 175 353028, 353045, 353112, 353118, 354 BD Falcon 175 Tray 5
Corning 175 3292, 431079, 431080, 431085, 43130 Corning 175 Tray 5
Nunc 175 159910, 159920 Easy Flask 175 Tray 5
Nunc 175 156502, 178883, 178893 Nunclon 175 Tray 5
TPP 150 90150, 90151 TPP 150 Tray 5
* For older style trays that require disks to hold vessels, see the "Help | Unavailable Trays" menu in the main window.

Figure 8-6. Find Vessel for Upcoming Scans

- Type: Flask, Microplate, or Dish brand name
- Area (Flasks and Dishes): Area refers to the growth area for a flask or dish.
- Wells (Plates): Number of wells in the plate
- Catalog #'s: Different catalog numbers can be available for a single flask, dish or microplate type depending on cap type, packaging, etc.
- Flask/Plate/Dish: Common name for the flask, plate or dish. This is the name that will appear in the Vessel Type pull-down menu.
- Tray Type: Indicates the Tray Type that should be selected for the desired flask or dish. There is no Tray Type column under the Plates tab because all plates use the Microplate Tray type.

Unavailable Trays

New versions of several IncuCyte™ trays have been issued since the original trays were released. Updated versions include trays 5, 7 and 9. The original trays 5, 7 and 9 incorporated the use of Spacer Disks in order to accommodate a wider variety of vessels. The old style versions of these trays are no longer being sold; however, some Users might retain these trays from their original purchase. The Trays 5, 7 and 9 listed under the Tray Type Find search (Figure 8-6) represent the new Tray Type versions. Information regarding the older versions of these trays can be accessed by clicking on Help, Unavailable Trays at the top of the IncuCyte™ software screen.

Triple-Wide Trays

Tray Types 10 and 11 are triple-wide trays, and a single triple-wide tray occupies the entire IncuCyte™ drawer. Therefore, only one of these trays can be used at any given time. See Figure 8-7.

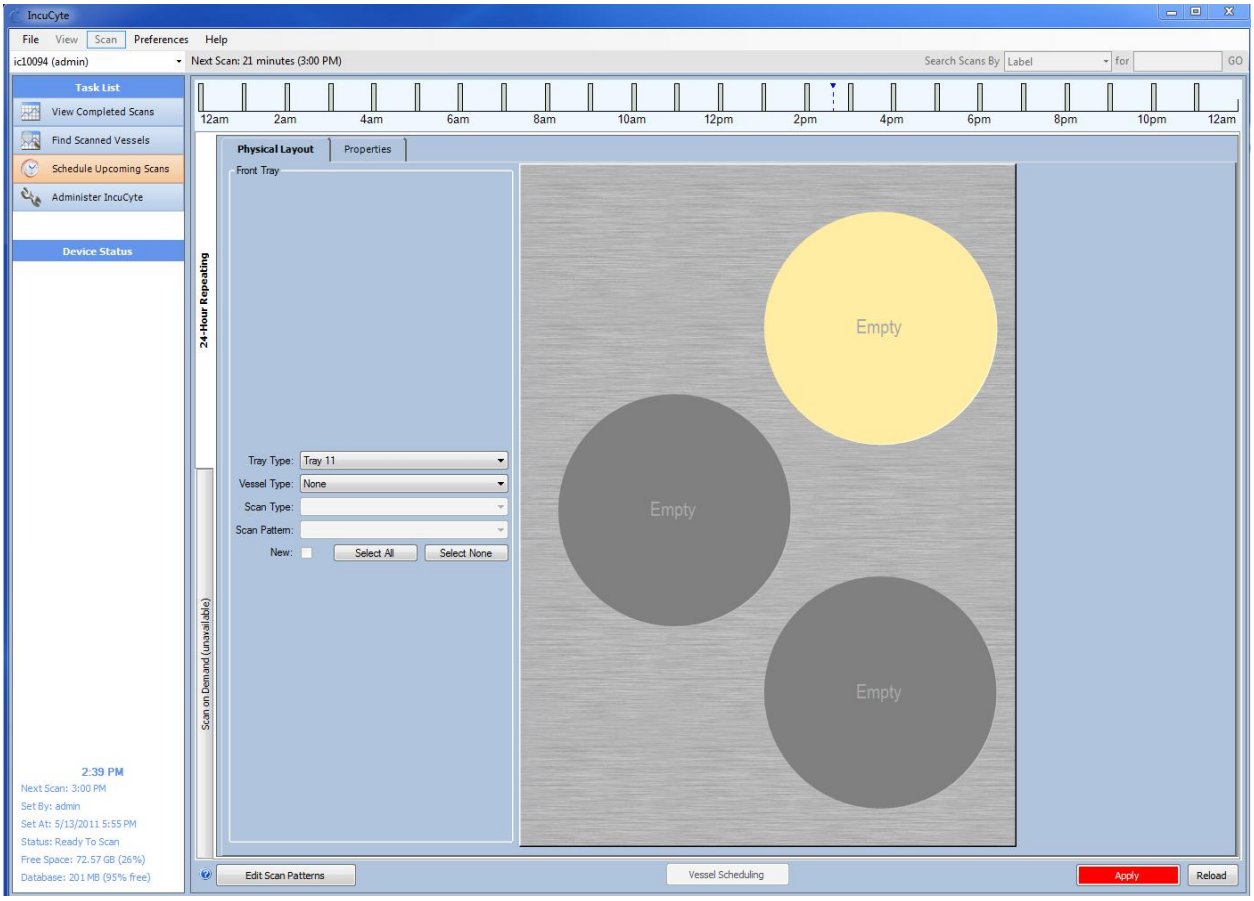


Figure 8-7. Tray Type 11: Because this is a triple-wide-tray, the IncuCyte™ can only accommodate one tray at a time.

Because the triple-wide tray will require the entire IncuCyte™ drawer, any vessels that have already been selected in regular-sized trays will be eliminated if a triple-wide tray is subsequently selected. Under these circumstances, a warning window will appear. Click on Yes to continue with the selection of the triple-wide tray, or click No to cancel.

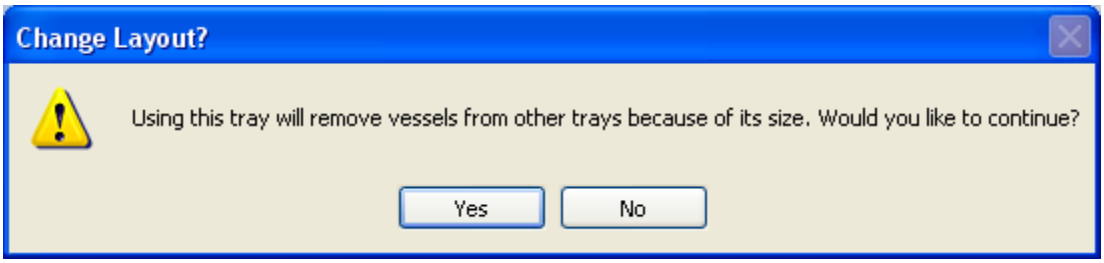


Figure 8-8. The Triple-Wide-Tray Warning Window

Adding Vessels to the Tray

A tray type must be selected, and then one or more vessel spaces within that tray type must be further selected in order for the Vessel Type menu box to become active. See Figure 8-9.

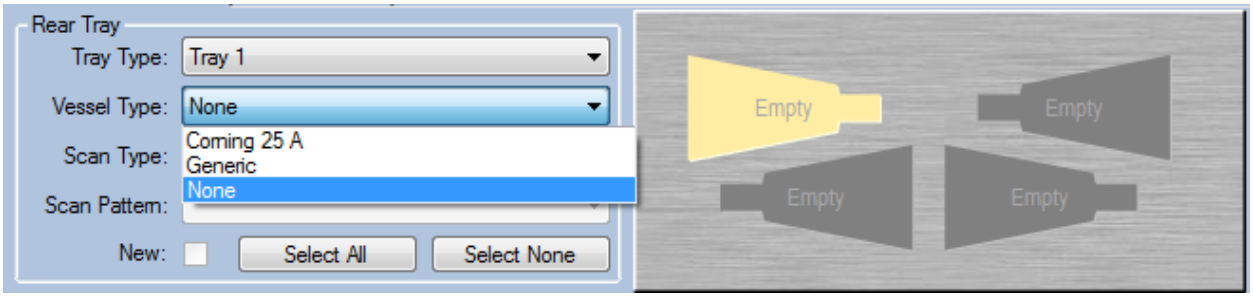


Figure 8-9. Vessel Type pull-down menu. The top, left space has been selected. Selected vessel spaces are highlighted yellow. The Vessel Type box is now active, and the option for that space can be displayed.

Use the mouse to click on a vessel space to highlight/select that space. If the mouse is clicked outside of the highlighted space, the space will become un-highlighted. The space can be selected again with the mouse. Vessels can only be added to highlighted spaces. Two or more spaces within a single tray can be simultaneously highlighted by clicking on one or more spaces, holding down the SHIFT or Ctrl key, and then selecting the next space(s) with the mouse. Alternatively, all spaces can be simultaneously highlighted by pressing the Select All button.

To add a vessel, select the Vessel Type pull-down window. This menu will list all compatible vessels by their common names. Choose the desired vessel, and it will populate all highlighted spaces in the tray.

Select All

Activate/highlight all spaces.

Select None

Deactivate/unhighlight all spaces.

Remove Vessel

To remove a vessel, right click on it and select Remove Vessel.

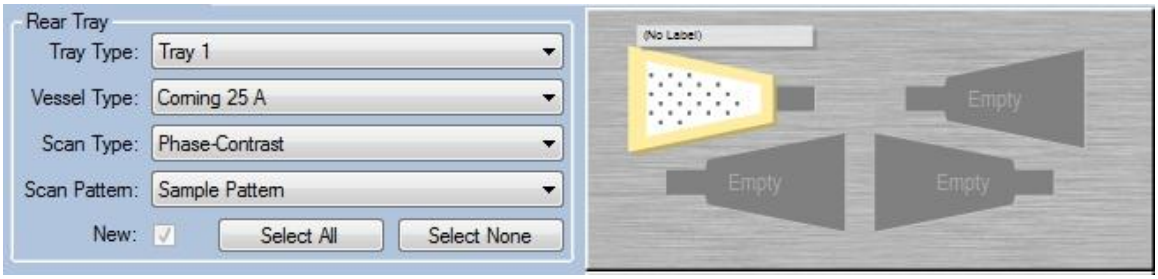


Figure 8-10. Upcoming Scans Tray after adding a flask.

Clicking the Select All button will highlight all spaces in a tray. If a vessel type is then chosen, that same vessel type will be added to all the spaces. If multiple spaces have been simultaneously selected using either the SHIFT or Ctrl key, these spaces will also be assigned the same vessel type. To choose different vessel types within a single tray, each must be added individually.

The Vessel Type pull-down menu includes an option for Generic. This option is available for those instances when a specific vessel desired by the User is not available through the vessel pull-down menu. Under these circumstances, add the desired vessel to the IncuCyte™ microscope and then select Generic within the software. A generic vessel image will appear in the tray. The IncuCyte™ will attempt to obtain the best images possible from the generic vessel; however, because the IncuCyte™ is programmed to optimize imaging according to the measurements of each specific vessel type, it is possible that the images obtained through the Generic option might be of a slightly lower quality.

Selecting Scan Type

For some vessels, or with the IncuCyte™ FLR, the User may choose a specific scan type:

- Phase-Contrast ImageLock™ Mode: Essen ImageLock™ plates can be scanned in ImageLock™ Mode. See Section 8.5.10, page 85.
- Fluorescence & Phase-Contrast Mode: Vessels can be scanned in Fluorescence and Phase-Contrast Mode with the IncuCyte™ FLR. See Section 8.5.2, page 66.
- Fluorescence and Phase-Contrast ImageLock Mode: ImageLock™ plates can be scanned in Fluorescence & Phase-Contrast ImageLock™ Mode with the IncuCyte™ FLR. See Section 8.5.11, ImageLock Mode FLR, page 86.

FLR

8.5.2 Setting Vessel Properties FLR

With the IncuCyte™ FLR, the default scan type is Fluorescence & Phase-Contrast. The User may also select to scan a vessel in Phase-Contrast mode only.

- Fluorescence & Phase-Contrast

  If Fluorescence & Phase-Contrast is selected, the vessel will be scanned both fluorescently and in HD mode.

  Fast Fluorescence allows for faster imaging and analysis by creating smaller images with no loss in sensitivity and is recommended for most applications. In the Properties tab, select the Fast Fluorescence checkbox. See below for more information.

- Phase-Contrast

  If Phase-Contrast is selected, the vessel will only be scanned in HD mode.

Fast Fluorescence

Users are encouraged to use the Fast Fluorescence (FF) acquisition mode because it provides some very important performance advantages. Perhaps the most important attribute of FF is the fact that the overall acquisition time is approximately 50% faster. This leads to smaller temperature rises for the same amount of vessel coverage. Another important advantage to using FF is the fact that the amount of time a specimen is illuminated in fluorescence is reduced by almost a factor of three. For specimens and assays sensitive to photobleaching, this is a huge reduction over a standard fluorescence experiment. Finally, FF images are nearly four times smaller than standard, resulting in faster image load times, exports and archives. Processing of fluorescence analysis jobs is also nearly four times faster than standard fluorescence analyses. And because FF images are smaller, the IncuCyte controller disk usage is also reduced dramatically.

In order to maintain the same sensitivity as standard fluorescence images, the tradeoff that FF makes is in the area of image resolution. FF images have less spatial resolution than standard fluorescence images, which means that the very finest spatial features that can be detected in standard images are observed with less detail in an FF image. For assays where high throughput is preferred over high content, the advantages of FF far outweigh the resolution afforded by a standard fluorescence scan. One example is a nucleus counting assay, where the FF resolution is more than adequate to detect cell nuclei.

NOTE: Only fluorescence images are acquired at reduced resolution using FF; phase images are still acquired at full resolution.

8.5.3 Setting Scan Patterns

Overview

The scan pattern determines the pattern(s) and location(s) of images that will be obtained within a vessel. After the vessel type has been selected, the Scan Pattern menu box becomes activated for that particular vessel. The Scan Pattern menu box will only be active when a vessel is highlighted, and the Scan Pattern selected will apply only to that one vessel. If two or more vessels are highlighted simultaneously, either using the SHIFT/Ctrl keys or by choosing the Select All button, then the selected Scan Pattern will be applied to all highlighted vessels. The Scan Pattern is indicated within each vessel as a pattern of green dots. A default Sample Pattern will be available for each vessel type when the IncuCyte™ arrives. Initially, this will be the only scan pattern available. However new scan patterns can be created by the User by selecting a vessel and then clicking on the Edit Scan Patterns button located at the bottom, left corner of the Schedule Upcoming Scans screen (the Edit Scan Patterns button is visible in Figure 8-5). This will bring up the Scan Pattern Manager window, Figure 8-11

The Edit Scan Patterns button can also be selected when no vessels are highlighted, but in that case the Scan Pattern Manager window will contain a random vessel that is not necessarily the one desired. New Tray Types and Vessels can be selected from within the Scan Pattern Manager window.

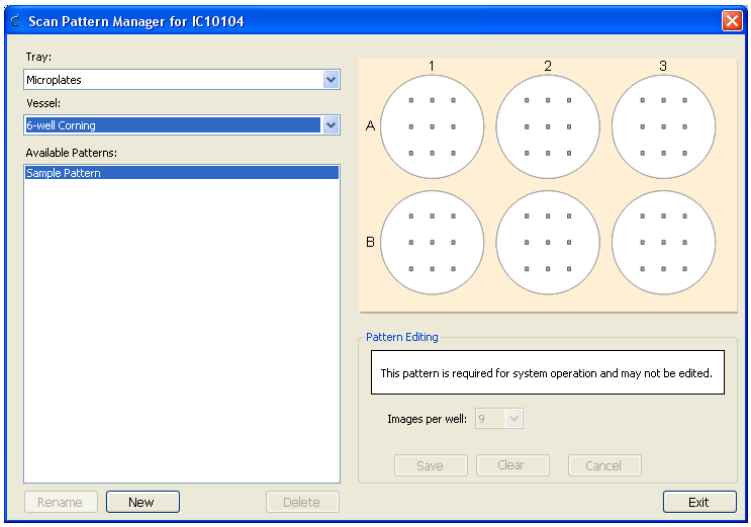


Figure 8-11. Scan Pattern Manager Window

Create a New Scan Pattern

The Scan Pattern Manager window is shown in Figure 8-11. A large view of the selected vessel will be displayed with the scan pattern indicated as green dots. The selected tray is indicated in the Tray menu box, and the selected vessel name is displayed in the Vessel menu box. The manager window will automatically open to show any vessel that was previously highlighted in the tray. However, a different vessel can also be selected using the Tray and Vessel pull-down menus.

The first time the manager window is opened, it will display the Sample pattern. The Sample pattern CANNOT be deleted or renamed. In this case, it is the only pattern available, so the Rename, Delete and Clear Pattern buttons are inactive as in Figure 8-11 . To create a new Scan Pattern, click the New button in the manager window. A window will appear requesting a name for the new pattern. Enter a name and click OK. Now the desired vessel will appear with no scan pattern. The only active selections will be the Clear and Cancel buttons and the Images per Well pull-down menu (Figure 8-12).

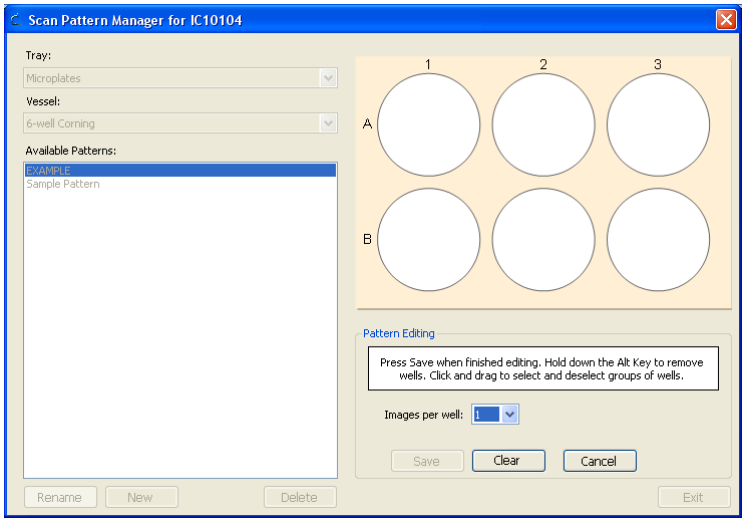


Figure 8-12. Making a new Scan Pattern

Select the number of images to be scanned per well or sector. Then, left-click on the empty well(s)/sector(s) to which the pattern will be applied in the vessel image above the Pattern Editing Box. The scan pattern will appear within the selected area. To remove the pattern from a well/sector, left-click on it. The desired number of images will appear in all wells/sectors that have been selected. Choosing a different image number will change the image number in all wells already containing a pattern.

NOTE: It is NOT possible to have different scan patterns between different wells/sectors of a single vessel.

Right-click over a well to enter/remove a scan pattern from multiple wells or sectors at once (Figure 8-13).

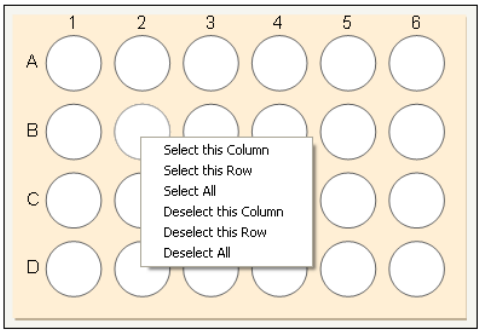


Figure 8-13. Right-click to select or deselect a Scan Pattern by All, Row or Column.

Alternatively, multiple wells can be selected by clicking and dragging with the mouse. The selected number of images per well/sector will appear in each well or sector. The scan pattern can also be removed by clicking and dragging while holding down the Alt key. See Figure 8-14. After a new scan pattern has been selected, the Save button will become active. Click it to save the new pattern.

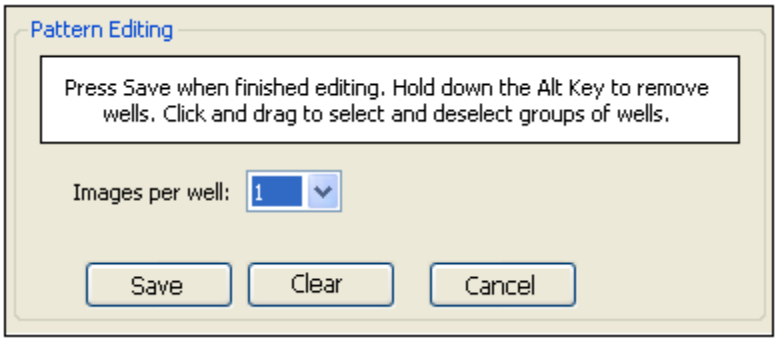

Pattern Editing
Press Save when finished editing. Hold down the Alt Key to remove wells. Click and drag to select and deselect groups of wells.
Images per well:
1
Save
Clear
Cancel

Figure 8-14. Close-up of the Pattern Editing box within the Scan Pattern Manager. Note the instructions for Pattern Editing.

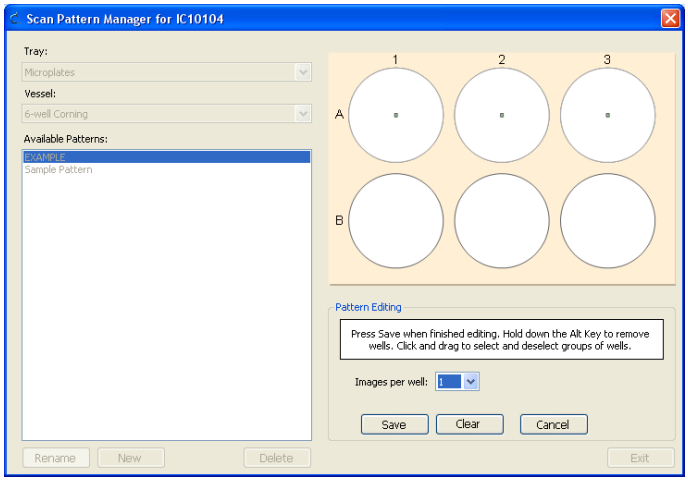


Figure 8-15. Scan Pattern Manager Window before saving

Once a new scan pattern has been created, it can be renamed or deleted, so the Rename and Delete buttons will become active for the first time. Remember that the Sample Pattern cannot be renamed or deleted.

After the new EXAMPLE pattern has been saved, it will appear as an active, selectable option in the Available Patterns box. When finished creating new pattern(s), press Exit to close the Scan Pattern Manager window.

> The new EXAMPLE pattern can be Renamed or Deleted. The Sample Pattern cannot.

The newly created Scan Pattern can now be selected under the Physical Layout tab, using the Scan Pattern pull-down window. See Figure 8-16.

It is possible to set scan patterns that generate an extremely large number of images per well. For instance, up to 121 images are available per well of a 6-well plate. There may be times when a large number of images are required. However, more images means longer scan lengths and more data to store. Therefore, be judicious about selecting scan patterns and use the minimum number of images that will provide representative data.

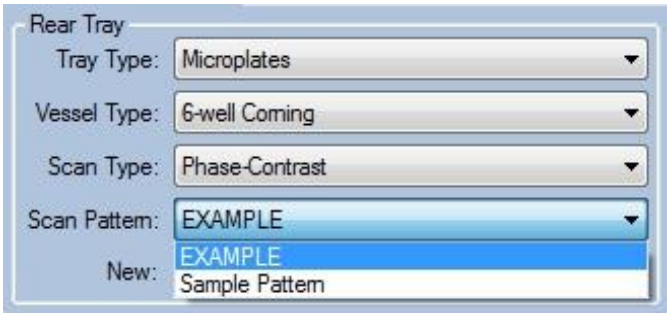


Figure 8-16. The EXAMPLE Scan Pattern is now available in the Scan Pattern pull-down menu.

> A Scan Pattern created for a specific vessel type will be available only for that particular vessel type. For example, if a scan pattern is created for a Corning® 75cm^2 flask, it will NOT be available for a Becton Dickinson® 75cm^2 flask - even though both are 75cm^2 flasks.

> A scan pattern CANNOT be deleted if it is currently selected in the pull-down menu and displayed in the tray, or if it is saved as a Layout. Saving Layouts will be discussed further in Section 8.5.13 (page 86).

EX

8.5.4 Setting Scan Patterns EX

To create scan patterns in the EX, navigate to the Administer IncuCyte™ Task Bar. See Figure 8-17. New Scan Patterns can be created and edited by EX

Users at the Administrator permission level, but they CANNOT be deleted. Scan Patterns can ONLY be deleted by the automated cell handling system.

NOTES:

In the IncuCyte™ EX, the Administer IncuCyte™ Task Bar is ONLY available at the Administrator permission level.

Scan Patterns in the EX can be created and edited, but they CANNOT be deleted through the IncuCyte™ EX local interface. This is true even for Users at the Administrator permission level.

To create a new Scan Pattern, first select the desired Vessel Type. See Figure 8-18.

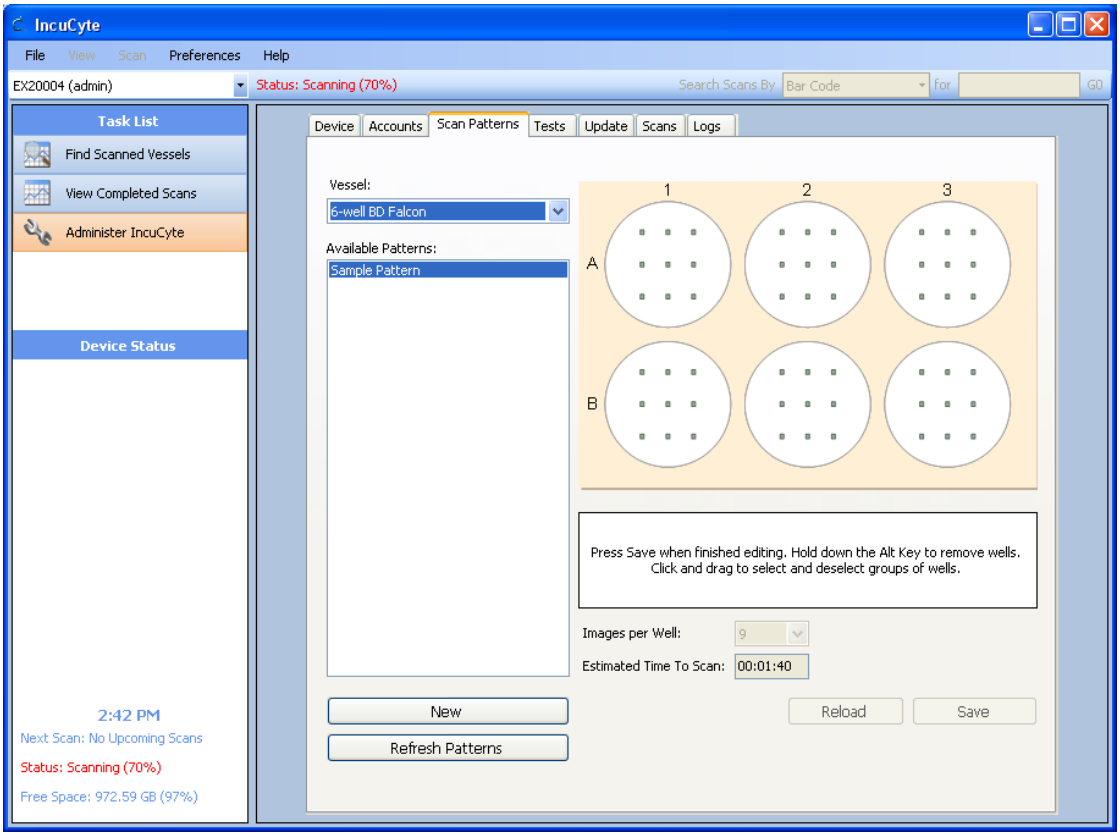


Figure 8-17. The Scan Patterns Tab under the Administer IncuCyte™ EX Task Bar

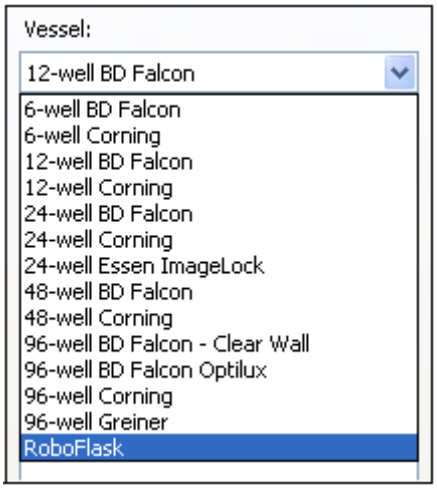


Figure 8-18. Select the Vessel Type for the new Scan Pattern in the EX.

A single Sample Pattern will be available for each Vessel Type by default. However, additional Scan Patterns can be created as required. The process by which new Scan Patterns are created is basically the same as for the Standard Model IncuCyte™ (Section 8.5.3, page 67). Existing Scan Patterns can be edited by highlighting the desired Scan Pattern and then selecting a new pattern.

The estimated scan length for each Scan Pattern is indicated in the Estimated Time to Scan window. In Figure 8-19, the estimated scan length for the selected Scan Pattern is 1 minute and 20 seconds.

IMPORTANT: Changing the Scan Pattern also affects the scan length of the target vessel. It is important to consider that significantly increasing the scan length of your own vessel could negatively impact the schedules of other, unrelated vessels.

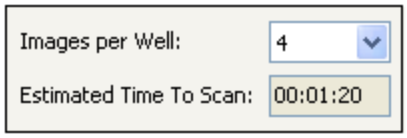


Figure 8-19. Estimated Time to Scan

Press the Refresh button to insure that you are viewing the most up-to-date set of scan patterns for the selected vessel. It is recommended to always press the Refresh button before assuming the existing pattern is still accurate. Scan patterns can be changed by other Users, and this will not be apparent unless the Refresh button has been selected.

Press the Reload button to return to the last scan pattern that was previously saved.

8.5.5 Setting Vessel Properties

After completing the Physical Layout, select the Properties tab. This Section facilitates entry of vessel properties such as Cell Type, Label, etc. Vessels selected in the Physical Layout screen will appear in the properties screen.

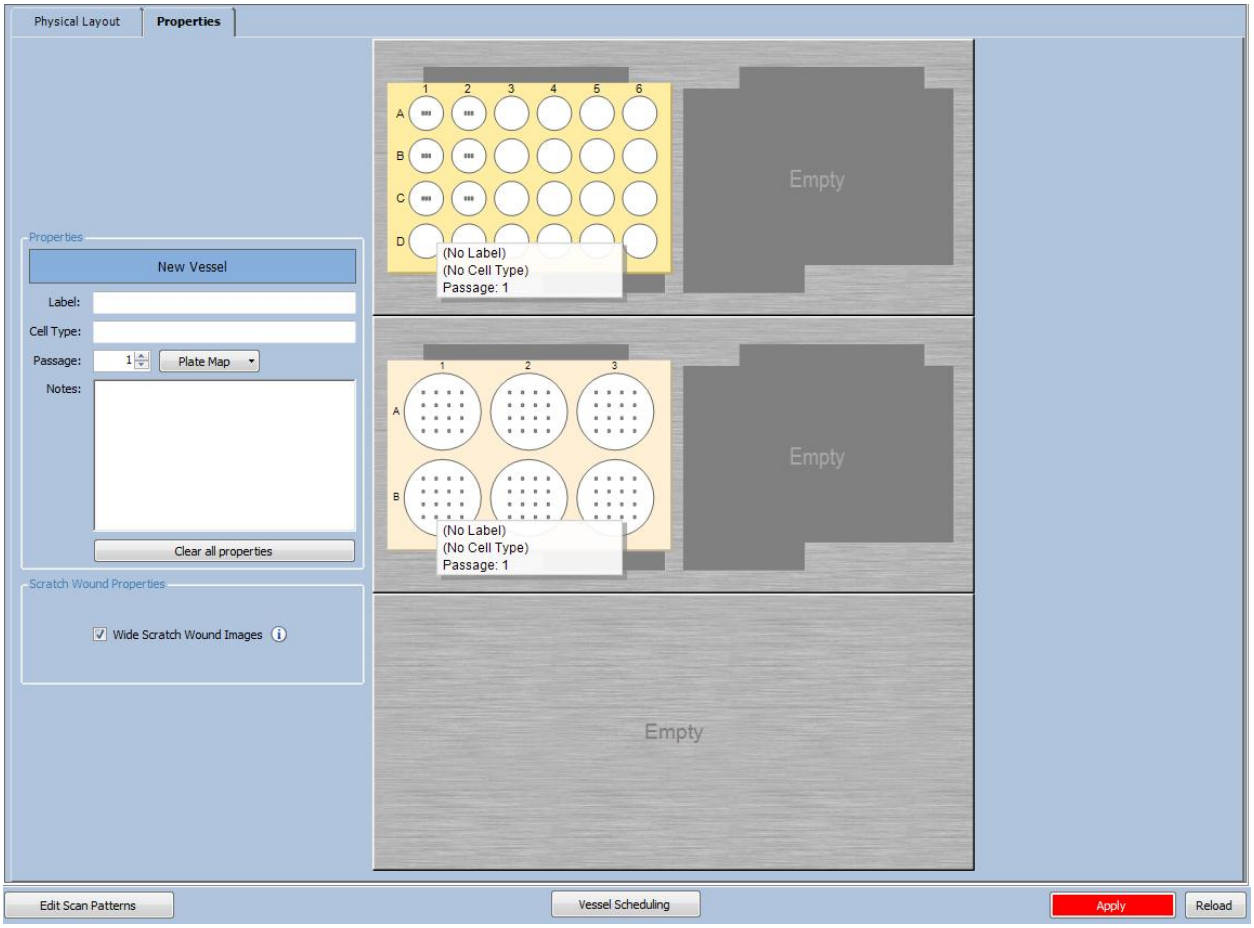


Figure 8-20. Upcoming scans with a New vessel

In Figure 8-20 there were no previous vessels of this type (same brand of 6-well plate) in this location for the previous scan, so the Properties Screen indicates that the selected vessel is New and the Label, Cell Type and Passage boxes are active. The Type box will be inactive unless the selected vessel is an Essen ImageLock™ plate (see Section 8.5.10 [page 85] for more information on ImageLock™ plates). The name of the current User and the time/date automatically appear in the Notes box. If the same vessel type in the same position was already present for a previous scan, then the Properties Screen indicates that it is an Existing Vessel (Figure 8-21).The New box is active, while the Label, Cell Type, Passage and Notes boxes are inactive and unchecked. To change the properties of an Existing Vessel and start a new scan series, check the New box. This will activate the previously inactive windows. Any previously existing text within these windows can be edited or deleted. Every time new

material is added to the Properties Section, the new User name, date, and time will appear automatically in the Notes box.

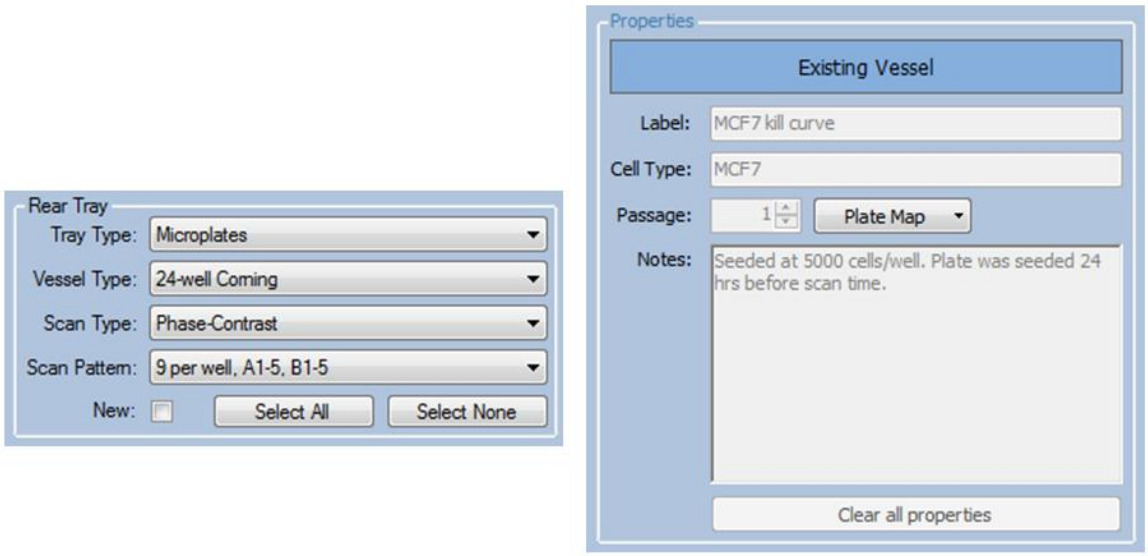


Figure 8-21. An existing vessel in the Physical Layout (left) and Properties (right) tabs of the Schedule Upcoming Scans screen.

Label

Name of the vessel

Cell Type

Cell type used in the vessel

Passage

Passage number of the cells can be selected by scrolling up and down, or a number can be typed directly into the passage box.

Plate Map

Use this button to open the Plate Map Editor and create a plate map for the vessel (microplates only). Once a plate map has been created and applied to a vessel, a small plate map icon will appear next to the plate map button. Hovering over this small icon will provide additional information for the applied plate map.

Notes

Any extra information desired.

In the View Completed Scans screen, vessel properties appear in a tab to the right of the screen, under the Selected Vessel Layout, and in front of each vessel

on the screen. The Properties can also be displayed in the View Completed Scans screen; however, this is an optional feature. The properties (if any) that are displayed for the completed scans can be adjusted using the View pull-down menu at the very top of the screen. The View menu will only be active on the View Completed Scans screen. See Section 8.7.1 (page 124).

EX

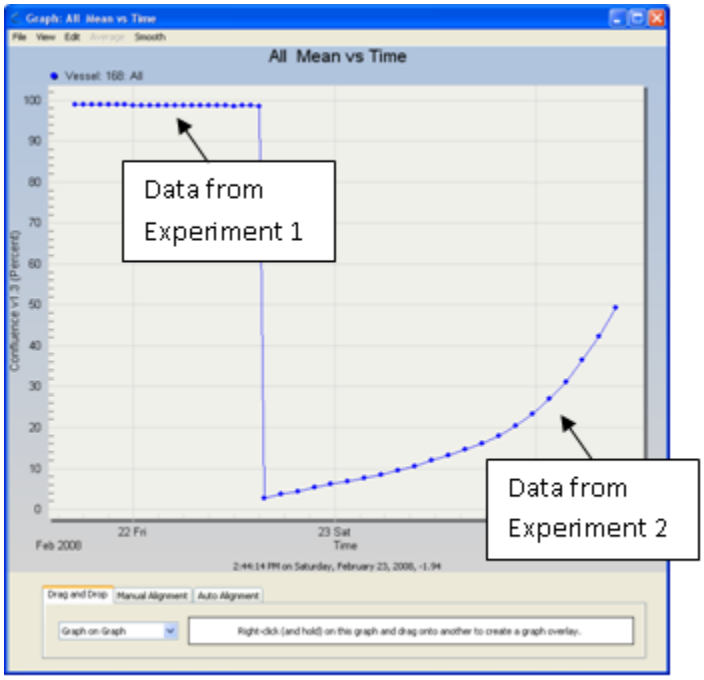


Figure 8-22. The New box was not checked at the beginning of a scan for a new vessel (Experiment 1). Therefore, when the flask is graphed, the graph for the previous vessel (Experiment 2) is connected to the graph for the new vessel.

8.5.6 Setting Vessel Properties EX

Vessel Properties CANNOT be set through the IncuCyte™ EX local interface. Rather, they must be set through the automated cell handler. The automated cell handler assigns values to the built-in IncuCyte™ vessel property windows such as Cell Type, Label, etc. However, the EX offers two additional tabs (Vessel and Scan), each of which offers a maximum of 5 Custom Vessel Fields and 5 Custom Scan Fields respectively.

Custom Vessel Fields are vessel-wide properties that do not change from scan to scan. An example would be “Seeding Date” (9 March 2008).

Custom Scan Fields are properties that do change from one scan to the next. An example would be the “ACH (automated cell handler) Scan ID” (Scan Job # 564).

See Figure 8-23. A single custom Vessel (Seeding Date) and a single custom Scan (Job # 564) have been assigned. The remaining 4 custom fields remain empty (Not Currently Set) for both the Vessel and Scan fields.

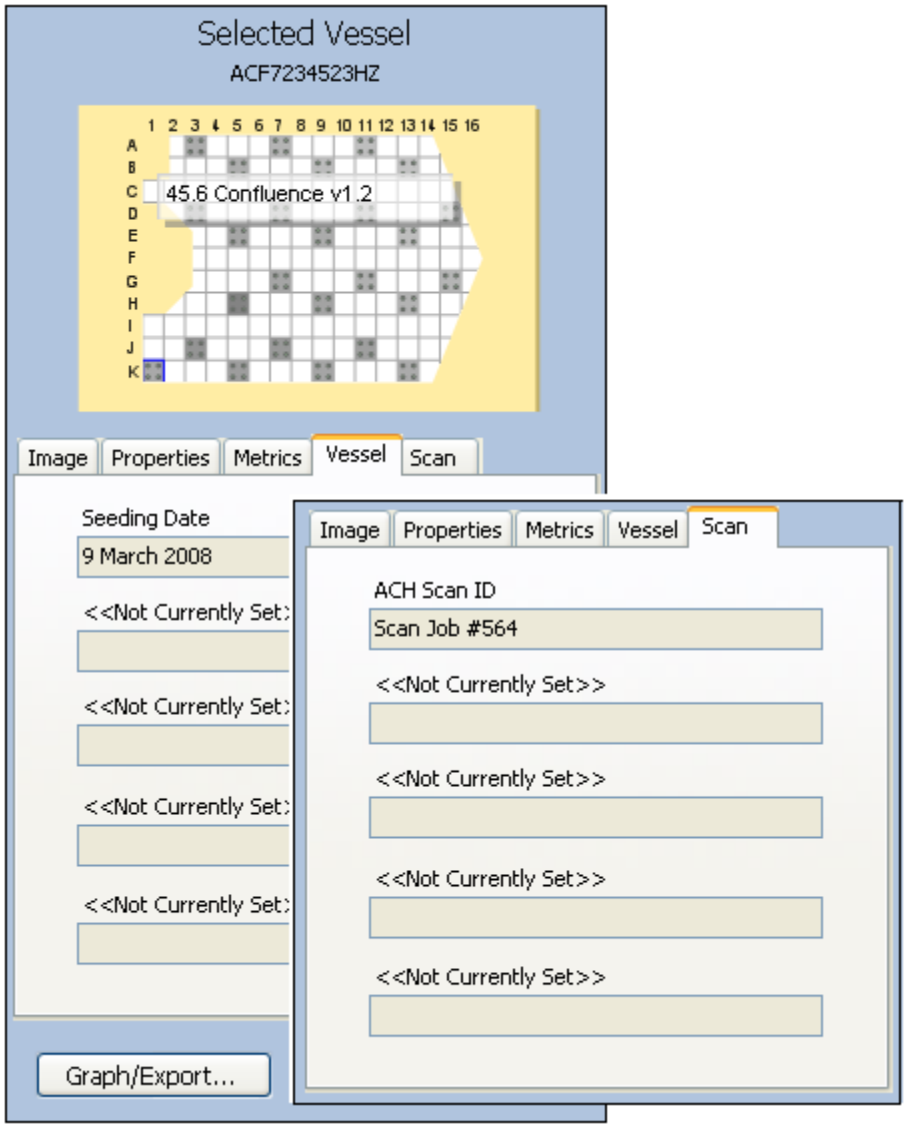


Figure 8-23. The EX Vessel and Scan Tabs

8.5.7 Setting Scan Times

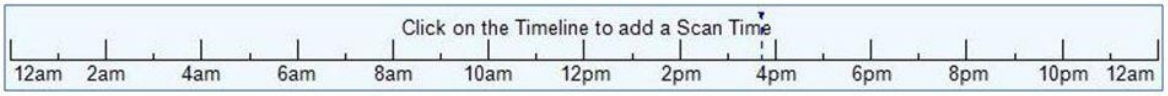


Figure 8-24. Timeline with no scan times set and a current time of ~3:40pm (noted by a dotted blue line).

Setting a Scan Start Time

To select a scan start time, left click on the Timeline. A gray Scan Bar will appear on the Timeline at that site (Figure 8-25). The exact scan start time can be visualized by positioning the mouse over the Scan Bar. The start time and estimated scan length will appear in a yellow box.

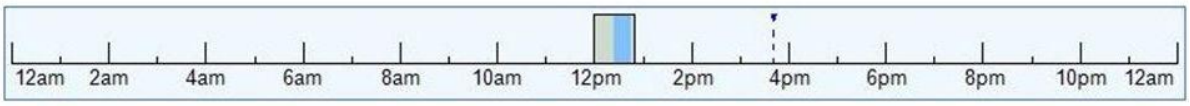


Figure 8-25. Timeline with one scan bar starting at 12 pm. The scanned vessels are separated by a cooling period.

The Scan Bar can be moved up and down the Timeline by left clicking and dragging it. Fine adjustments in the start time can be made by right clicking on the Scan Bar. A pull-down menu will appear as in Figure 8-26. Selecting -1 Min, -5 Min, +1 Min or +5 Min will reset the Scan Bar by the corresponding lengths of time.

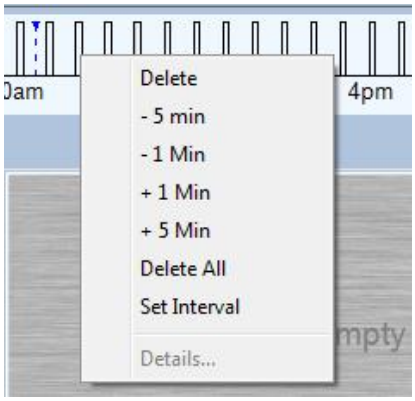


Figure 8-26. Scan Bar Pull-Down Menu

This same pull-down menu can be used to delete the selected Scan Bar or delete all scans on the timeline.

To set multiple scan times, simply set Scan Bars at all desired times.

Setting Scan Intervals

Frequently, it will be desirable to set up a series of scans to occur at a regular interval. Rather than setting each start time individually, a fixed scanning interval can be selected by right-clicking on the timeline and selecting Set Interval (Figure 8-26). This will open the Set Timeline Scan Intervals window shown in Figure 8-28. If an interval is desired that is not present as a selection within this menu, then the interval must be set manually by setting a Scan Bar at each selected start time as described in Setting a Scan Start Time.

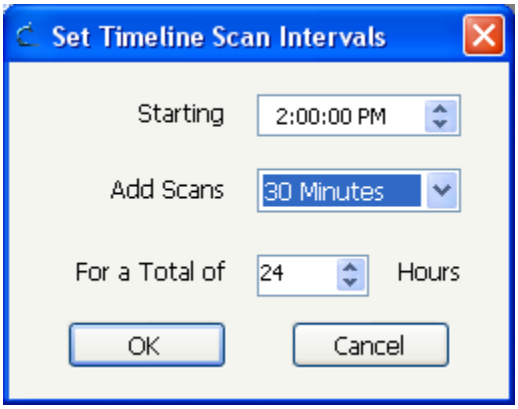


Figure 8-27. Set Timeline Scan Intervals

NOTE: Two scans CANNOT be set to occur at the same time.

Scan Duration

The duration during which scanning will occur can be set using the For a Total Of option. If a total of 24 hours is set, as in Figure 8-27. Set Timeline Scan Intervals, scanning will occur around the clock without interruption from one day to the next. If a shorter duration is selected, scanning will occur only during that duration (from start time to stop time). However, if the scan setting is not changed, the same scans will occur for the same duration on the next day, etc, until the scans are deleted from the instrument.

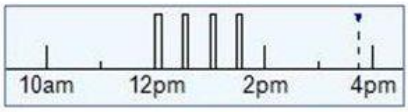


Figure 8-28. Scans set to start at 12pm, every 30 minutes, for a total duration of 2 hours.

In Figure 8-28, scans have been scheduled to start at 12pm, every 30 minutes for a duration of 2 hours. The instrument will scan each day between 12pm and 2pm until the scans are deleted.

Note that in Figure 8-28, a total of four scans will take place, with the final scan occurring at 1:30pm - NOT at 2pm.

There is a maximum amount of scan time recommended during any given time interval. If the number of set scans exceeds this amount, then a warning window will appear when the scans are applied. In this case, the number of set scans should be reduced.

Scan Length

The scan length is determined by the number of images that will be obtained during the scan. As a general rule, it is recommended that total continuous scan time not exceed 45 minutes. This is because the IncuCyte™ will warm slowly as a result of the scanning process, and prolonged scan times can result in a temperature increase of the instrument and, subsequently, of the incubator. Longer scan times can be set if the increase in temperature that might occur is not considered to be a critical factor. Generally speaking, larger incubators are more resistant to temperature changes than smaller ones, but the cells within the instrument will become warmer either way. To minimize temperature change, use the Vessel Scheduling feature (Section 8.5.8).

96-well plates have an increased initial scan length

96-well plates have a particularly small imaging area and, in order to generate a quality image, the camera must be very precisely located above each well. To ensure proper placement, the very first scan of a 96-well plate will require extra time (~50% total scan time) to assure optimum well/camera alignment. Subsequent scans will NOT require extra time. Whenever a scan is applied that contains 96-well plates, a pop-up window will appear reminding the User that the first scan will be longer by __ minutes (the exact number of minutes depending on the specific scan pattern). In order for the scan to proceed, the OK button must be selected, confirming that the User is aware of the increased scan time. This longer initial scan length ONLY occurs with 96-well plates.

HD

Note that the increased initial Scan Time for 96-well plates does NOT apply to the IncuCyte™ HD.

Scan overlap as a result of the longer, initial scan

The increased scan length required for 96-well plates must be taken into account when setting up the scan interval(s). It is possible that, even though all subsequent scans will be fine, the longer first scan could overlap with the beginning of the second scan. If this is the case, then the second scan will be skipped. Scanning will start again at the third scheduled scan time. If such an overlap will occur, a warning window will appear indicating that the second scan will be skipped. The second scan can be set to start after a somewhat longer interval, with subsequent scans occurring at the regular (shorter) interval. But if the scanning interval is offset in this way, it is important to remove the very first Scan Bar from the Timeline after it is completed. This is because, after the IncuCyte™ has finished the scans for the day, it will come back and start the scans set for the beginning of the next day. If the offset Scan Bar is not removed, the IncuCyte™ will include it in the next day's scan, thus disrupting the new interval and possibly causing another overlap.

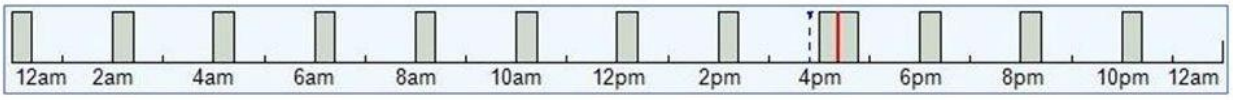


Figure 8-29. Scan overlap.

When scanning starts for the next day, the offset, initial scan time will still be on the Timeline causing an overlap (Figure 8-29). This overlap would prevent the scan from being set. However, if the scan times were not so long, the additional scan time would simply remain. The scan could proceed under these circumstances, but the 2-hour time interval would contain an extra scan between 4 and 6 pm (2 scans are clumped together at the 4 pm time point; Figure 8-30).

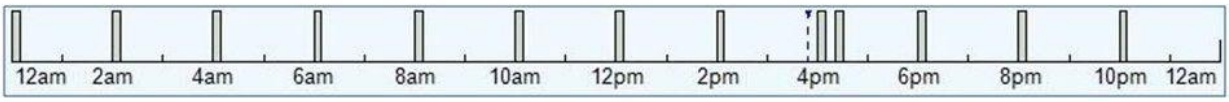


Figure 8-30. Scan overlap eliminated with shorter scan time.

FLR

Scan Length FLR

Fluorescence & Phase-Contrast scans are approximately twice as long as standard scans because the vessel will be scanned in both fluorescence and phase modes. Thus, each image can be viewed as a fluorescent or HD image. See Section 9.2, page 202.

NOTE: For a record of the temperature within an IncuCyte™ FLR, click the graph box at the bottom left of the screen, next to the current temperature reading in the Device Status box. See IncuCyte™ FLR Temperature History.

Scan Overlap

If two Scan Bars are set at the same time or if the Scan Bars are dragged so that they overlap, the offending Scan Bars will be highlighted in red. In Figure 8-29, there are two scan times to the left that overlap. If the User attempts to apply these scan times, a pop-up window will appear indicating that there is an overlap problem. Overlapping scans must be eliminated before scanning can occur.

After scan times have been set, click on the Apply button at the bottom of the screen to initiate the new scan schedule. A new window will pop up indicating that the changes made have been applied (i.e. saved). Now the IncuCyte™ is ready to start scanning. The time for the next scheduled scan will appear at the top of the IncuCyte™ screen.

IMPORTANT: The Apply button MUST be selected before new parameters will be saved and scanning will be initiated.

Processing Metrics and Scan Overlap

After scanning has been completed, the IncuCyte™ must process the data in order to generate images and corresponding metrics (% confluence and associated statistics). Processing of metrics will only commence after scanning is finished. Therefore, the results of the scan will not be immediately available after scanning is complete. The duration of metrics processing will depend upon the number of images obtained. If the between-scan interval is very short, then it is possible that the next scan will start before metrics processing is complete. In this case, the processing will be interrupted to enable the next scan. However, the interrupted processing will continue after scanning has been finished. If there is a series of such scans, then the processing will require extra time to catch up after completion of the final scan.

8.5.8 Vessel Scheduling

To avoid prolonged scans (greater than 45 minutes) or to customize the order in which vessels are scanned, click the Vessel Scheduling button at the bottom of the Schedule Upcoming Scans screen. The Vessel Scheduling window displays a list of all scheduled scans as a timeline and a list.

Change Scan Order

To change the order in which IncuCyte™ scans vessels, either (1) click and drag a vessel on the timeline into the desired position or (2) use the Move Before and Move After buttons to rearrange the scan order.

Add a Cooling Time

To add a cooling (idle) period between two vessels, the Auto-cool option is recommended. Auto-cool will find the most efficient vessel schedule possible with the current scan duration and interval. If a specific scan order or timing is preferred, click the Auto-Cool Settings button.

Use the Auto-Cool Settings window to adjust how Auto-Cool will rearrange the current vessel schedule. To minimize the number of scan groups and/or the length of the entire scan period, check the first box to allow Auto-Cool to rearrange the order in which IncuCyte™ scans the vessels. Check the second box to ensure all scan groups (groups of vessels scanned without interruption) begin at a set interval (on the half-hour, quarter-hour, *etc.*)

To manually insert cooling times, click the Add Cooling Time button and drag the cooling time block to the desired location on the timeline. To change the length of the cooling time, click the appropriate Cooling Minutes box in the list below the timeline and type in the cooling time (in minutes).

Cooling times appear as blue intervals on the timeline in the Schedule Upcoming Scans screen (See Figure 8-25. Timeline with one scan bar starting at 12 pm. in Section 8.5.7)

Related Notices

If adding/removing a vessel or changing its scan pattern/type changes the set vessel schedule, a warning will appear either on the scan timeline (overlapping scans) or next to the Vessel Scheduling button. If this warning appears, go back to the Vessel Scheduling window to view the revised schedule and edit it if necessary.

The Vessel Scheduling warnings refer to changes from the last *applied* schedule, not to any changes made after the Apply button was clicked.

Vessel scheduling may result in overlapping scans if the original scan interval is too short. In this case, change the length of the scan interval on the timeline in the Schedule Upcoming Scans screen.

SCHEDULING TIPS

Adding cooling times: During a cooling time, IncuCyte™ is simply idle. As such, you may open the instrument, add or remove vessels/trays from the instrument, feed cells, *etc.* while IncuCyte™ is cooling.

Viewing the vessel schedule: For the best view of the entire schedule, expand the Vessel Scheduling window horizontally. This will expand the timeline boxes so more information about each vessel is visible.

NOTE: Using the Auto-Cool option is recommended and allows for the greatest amount of scan time possible

8.5.9 Scan on Demand

In addition to the 24-Hour Repeating scan schedule, users also have the ability to scan in the Scan on Demand mode. Simplistically, this means that the IncuCyte™ can be used to scan individual vessels at times when the IncuCyte™ is not scanning. This includes both periods in between regularly scheduled scans in the 24-Hour Repeating scheduler, as well as in designated Cooling Times. To access the Scan on Demand scheduler, click on the vertical tab within the Schedule Upcoming Scans screen labeled Scan on Demand. However, all changes to the 24-Hour Repeating Schedule must either be applied to the IncuCyte™ by clicking the red Apply button, or reloaded to an unchanged state by clicking the Reload button before the Scan on Demand scheduler can be accessed. If changes are not applied or reloaded, the Scan on Demand tab will be unavailable.

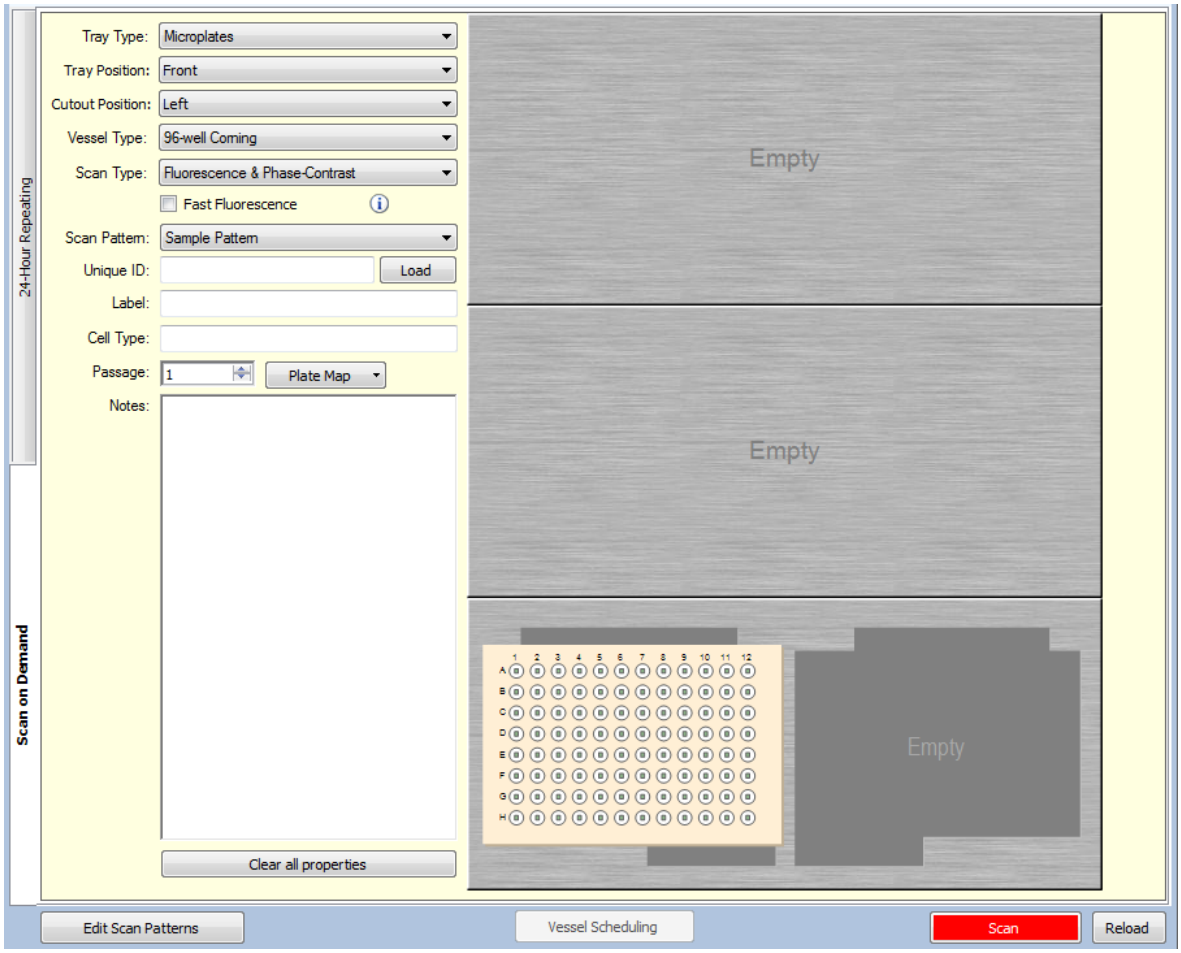


Figure 8-31. The Scan on Demand Scheduler.

To insert a vessel in the Scan on Demand mode, the Tray Type must be selected followed by the Vessel Type. This can be accomplished in exactly the same manner as it is in the 24-Hour Repeating mode. Because only one vessel can be scanned at a time in the Scan on Demand mode, the user also has to select the Tray Position (Front, Middle, Rear) in addition to the Cutout Position (Right or Left in the example in Figure 8-31). All three tray positions and either cutout position can be used. The same is true for all of the tray types. In addition, the user must swap out the correct trays within the IncuCyte™ microscope system itself, paying special attention to the location and description of the vessels that are removed so that they can be correctly replaced once the Scan on Demand has been finished.

In addition to a Label, users can also supply a Unique ID to each vessel. This Unique ID can either be generated manually by typing or pasting information into the Unique ID field, or a Unique ID correlating to the current year/day/time will be assigned to the Scan on Demand vessel. This information can be used to link subsequent scans together. However, it is important to note that if the user fails to type in, or select (using the load button) the correct Unique ID, scans will not be linked together, and cannot be accomplished post scanning.

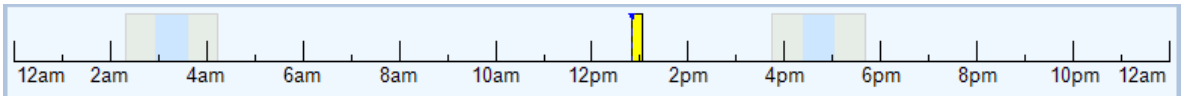


Figure 8-32. Scan on Demand time bar illustrating acceptable scan

Within the Scan on Demand scheduler, the 24-Hour Repeated Scan schedule (including all of the time bars) will be faded to the background, but will be clearly visible (See Figure 8-32). The current time will be indicated as in the 24-Hour Repeated scheduler, but will be followed by the Scan on Demand Time bar in yellow. Similar to the 24-Hour Repeating Schedule, the width of the time bar will correlate to the amount of time required to scan the vessel inserted into the Scan on Demand Tray.

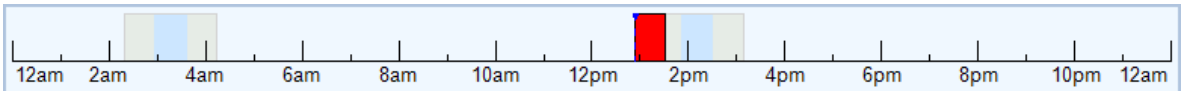


Figure 8-33. Scan on Demand time bar illustrating scan time overlaps with 24-Hour Repeating Schedule

Clearly, additional scheduling rules apply to the Scan on Demand scheduler in order to protect regularly scheduled scans from being skipped. These rules include:

- The Scan on Demand Time bar is typically yellow. If the Scan on Demand Time bar is Red as in Figure 8-33, this means that the Scan on Demand scan time overlaps with a scan time in the 24-Hour Repeating Schedule. Clicking the Red scan button will result in an error.

- The estimated time (indicated by the width of the yellow bar) to scan in the Scan on Demand mode is ≈20% longer than the estimated time in the 24-Hour Repeated scheduler. This additional

time is required so that the user has time to replace the Scan on Demand vessel with the 24-Hour Repeated vessel.

Scanning using the Scan on Demand Scheduler is initiated by clicking the red Scan button (Figure 8-31). For additional information, descriptions, warnings, and important notes regarding Scan on Demand, please read Section 7.1.4, Page 40.

8.5.10 ImageLock™ Mode

Essen BioScience manufactures a special 24- and 96-well plate type called the Essen ImageLock™ plate. These plates facilitate very precise, repeated imaging within each well. Under standard imaging conditions, slight variations can occur in the location of imaging from one scan to the next, resulting in small jumps between frames. When very precise, repeated imaging is required – for instance, when generating a movie – use the Essen ImageLock™ plate. By selecting an ImageLock™ plate and running it in ImageLock™ mode, the highest fidelity movie will be generated.

If an ImageLock™ plate is selected as the vessel type (under the Physical Layout tab), the Type box will be active (Figure 8-34). While Phase-Contrast ImageLock™ will be the default setting, an ImageLock™ plate can be run in either Phase-Contrast ImageLock™ or Phase-Contrast mode.

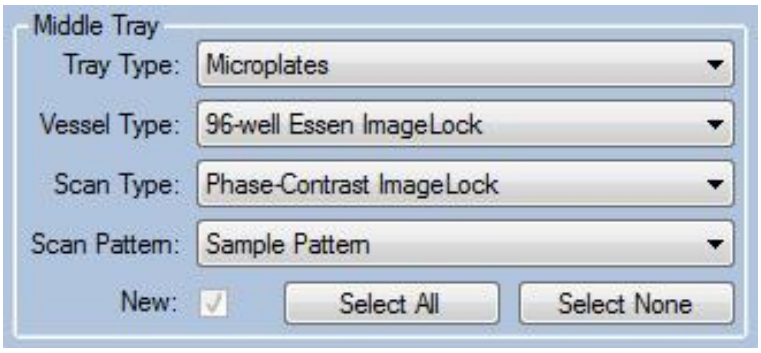


Figure 8-34. Running a Plate in ImageLock™ Mode

If an ImageLock™ plate is run in standard Phase-Contrast mode, the result will be comparable to that of a “normal” (non-ImageLock™) plate.

NOTE: It is recommended that ImageLock™ plates ONLY be run in ImageLock™ mode.

ImageLock™ Scan Length

Because the scanning process is so precise in ImageLock™ mode, the scan length will be longer than that for a plate run in Phase-Contrast mode.

ImageLock™ Scan Patterns

The scan patterns available for the ImageLock™ 24 and 96-well plates differ from those available for standard 24 and 96-well plates. A maximum of three images is possible per well, and the scan pattern is arranged in a straight line through the center of each well (Figure 8-35).

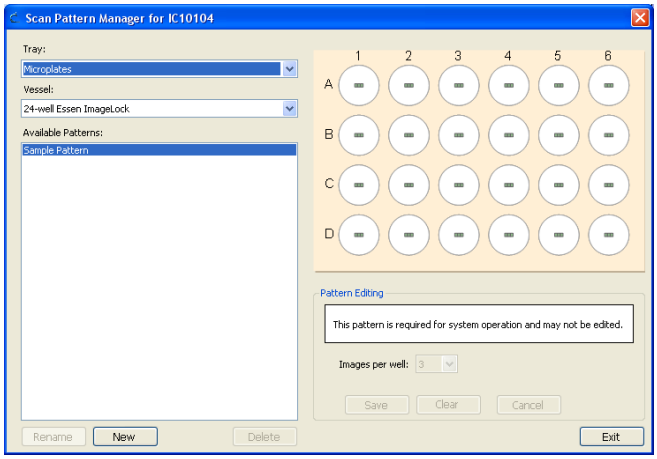


Figure 8-35. Setting Scan Patterns in ImageLock™ Plates. In the Sample Pattern, there are three images per well, and all three images are arranged in a straight line along the center of each well.

8.5.11 ImageLock Mode FLR

It is possible to perform fluorescence scans in ImageLock™ mode. If you have an FLR instrument, select Fluorescence & Phase-Contrast ImageLock™ from the Type pull-down menu.

8.5.12 Reload

Pressing the Reload button at the bottom of the Schedule Upcoming Scans screen will reset all settings (physical layout, properties, scan times, etc). back to the way they were prior to the last time the Apply button had been selected.

NOTE: When the Schedule Upcoming Scans window is open but idle (you are not actively scheduling a scan), the window will refresh every two minutes. This way, if someone applies a schedule change from a different PC, the revised schedule will automatically appear on your screen.

FLR

8.5.13 Layouts

Sometimes, the same Layout i.e. vessel type(s), scan pattern(s), etc. will be used on a regular basis. It would be convenient to save this Layout so that the vessels and scan patterns do not have to be set up every single time the configuration is required. A Layout can be saved in the Schedule Upcoming Scans screen by clicking on File at the very top left of the screen. Create the configuration to be saved. Select, Save Layout As from the File pull-down menu. This will bring up the Save Layout window (Figure 8-36. Save Layout EXAMPLE 2). Enter the

name of the new Layout. Save the Layout to add it to the Current Layouts box. To open/load an existing Layout, select Load Layout from the File pull-down screen. Highlight the desired Layout from the Load Layout window.

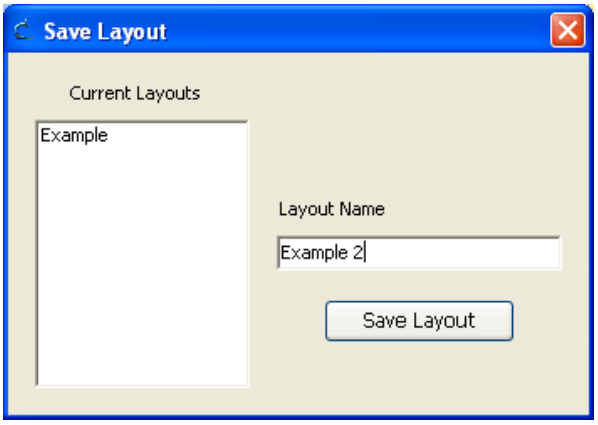


Figure 8-36. Save Layout EXAMPLE 2

An existing Layout can be deleted using the File pull-down menu.

8.6 Administer IncuCyte™

The functions available on the Administer IncuCyte™ screen will vary according to the Permission Level of the User (this topic is further discussed in Section, 5.3.2, page 21):

NOTE: We strongly recommend that ONLY individuals at the Administrator permission level implement changes to the controller time settings.

The Administer IncuCyte™ screen is only available to Administrators and Users (not to Guests). Administrators have access to all administrative functions, but Users have access to only a subset of functions. The Administer IncuCyte™ screen automatically opens to the Device tab. Figure 8-37 shows the screen available to Administrators, and Figure 8-38 shows that available to Users. The Administrator screen includes tabs for Device, Accounts, Tests, Update, Scans and Logs, and all features within each tab are active. At the User level, however, only the Device, Accounts and Logs tabs are present. Additionally, some of the features within these tabs are inactivated.

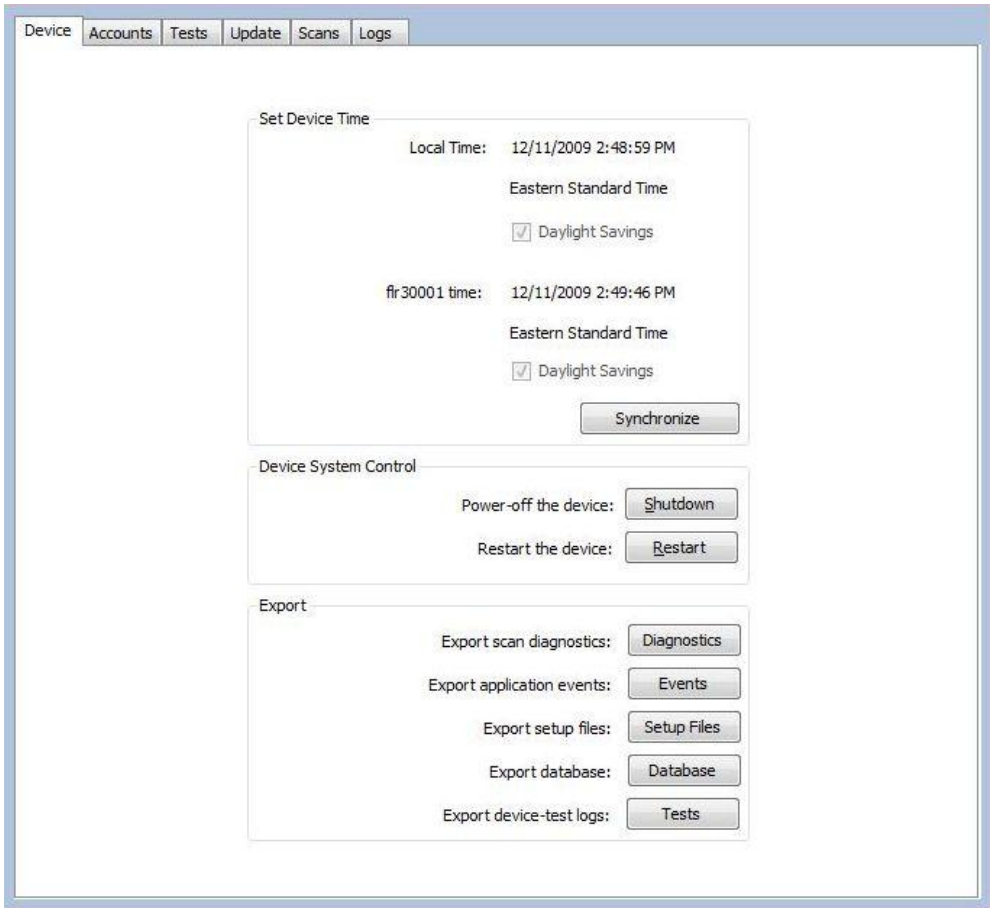


Figure 8-37. Administer IncuCyte™ at the Administrator Permission Level

EX

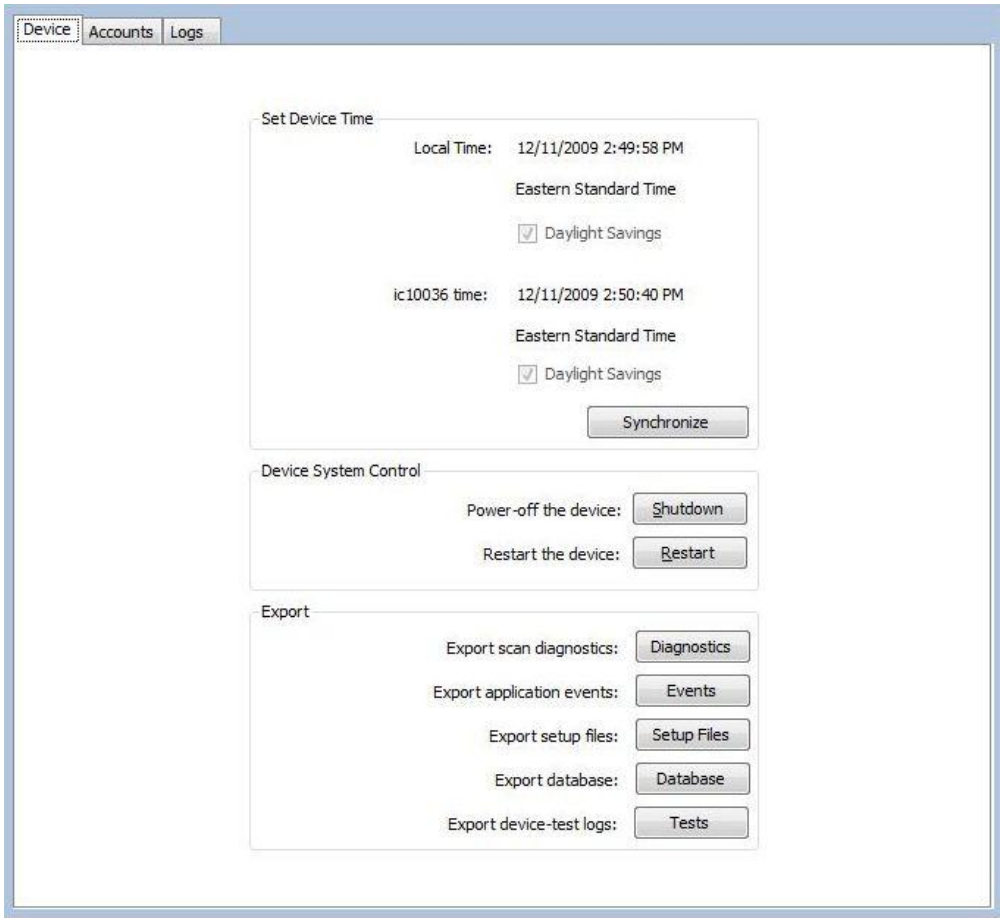


Figure 8-38. Administer IncuCyte™ at the User Permission Level

8.6.1 Administer IncuCyte™ EX

On the IncuCyte™ EX, the Administer IncuCyte™ tab is ONLY available to Users at the Administrator permission level (Figure 8-37). The complex nature of the scan schedules and the robotic component of vessel scanning necessitates strict control of administrative functions on the EX. We HIGHLY recommend that the number of EX Users with access to the Administrator permission level be kept to an absolute minimum. Ideally, only those individuals who interface with the automated cell handler would also have Administrator level IncuCyte™ access.

IMPORTANT: We highly recommend that the number of EX Users with access to the Administrator permission level be kept to an absolute minimum!

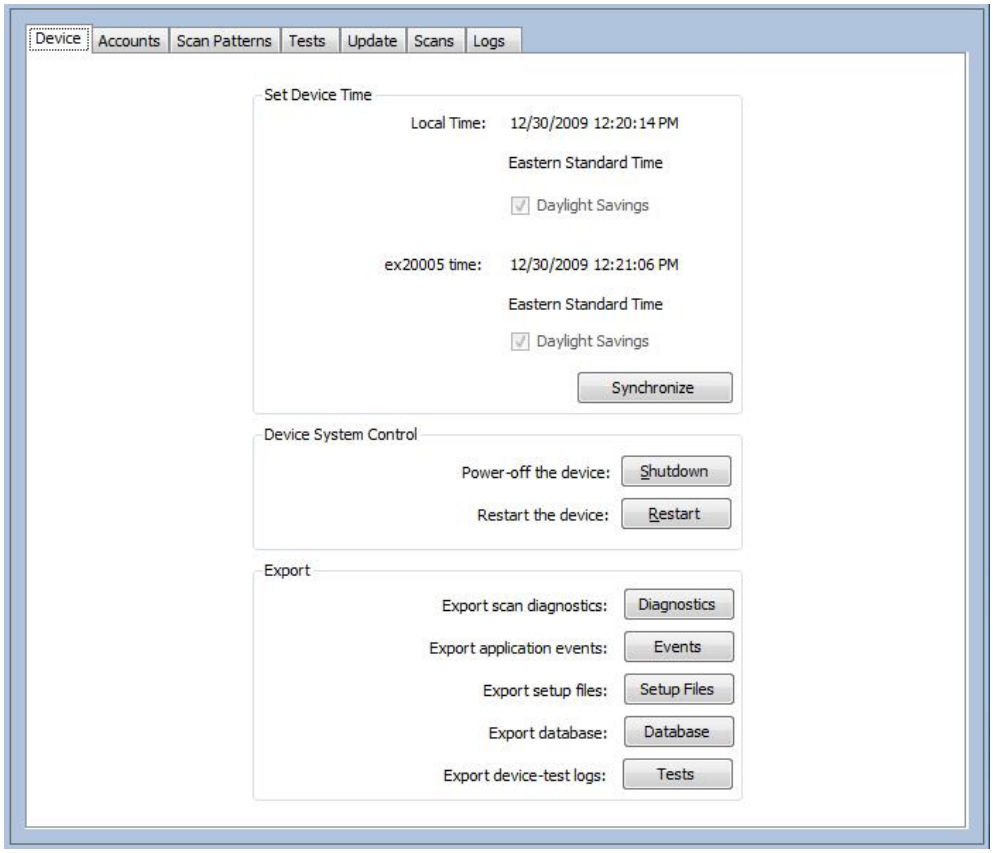


Figure 8-39. Administer IncuCyte™ EX

8.6.2 Device Tab

Set Device Time

The IncuCyte™ controller settings will be synchronized with the local computer at the time of installation. It is unlikely that any further synchronization will be required after this point. Should a disparity arise, however, the Set Device Tab can be used to adjust for these differences.

It is important to remember that, aside from being synchronized, the controller time settings should also be correct. Synchronizing the controller to an incorrect time setting can lead to unexpected scan results. Therefore, if a disparity between the IncuCyte™ controller and the local computer becomes apparent, the accuracy of the local computer settings should be confirmed prior to adjusting the controller settings. This is especially important if a single IncuCyte™ controller is being accessed by multiple individual computers.

NOTE:

Synchronization is ONLY required if a prompt is issued by the software. In this case, a prompt will appear following login (Figure 8-40 and Figure 8-41)

If you are prompted to synchronize, it is important to confirm that your personal computer has the right time and the correct settings.

Every time the IncuCyte™ software is launched, it automatically compares the Time Zone and Daylights Savings Time settings with the controller's settings. There is also a comparison between the controller time and the local PC time. If these settings or values are not in agreement, a window will be launched to this effect.

NOTE:

We strongly recommend that only individuals at the Administrator permission level implement changes to the controller time settings.

Synchronization is ONLY permitted when the device status is idle. For instance, synchronization cannot occur during a scan.

It is important to close the IncuCyte™ control software before making any changes to the time/time zone settings on your local computer.

Time Zone and Daylight Savings Time

If the Daylight Savings and/or Time Zone settings of the controller and the local computer are not in agreement, a message window will appear after login, but before the software is launched.

- **Time Zone**: In this case, the Time Zone on the local computer and the controller (server) for the IncuCyte™ are not properly synchronized. One of two messages will be displayed depending on whether you are at the Administrator (Figure 8-40) or User permission level (Figure 8-41).

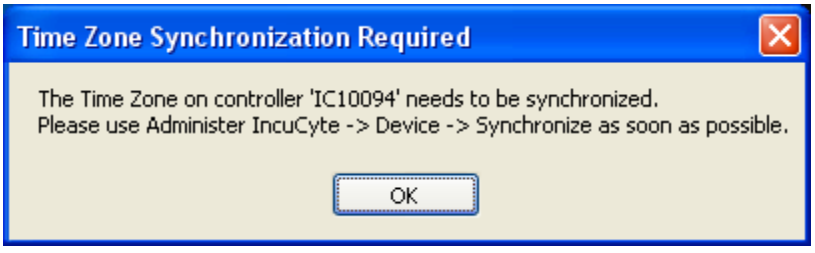

Time Zone Synchronization Required
The Time Zone on controller 'IC10094' needs to be synchronized.
Please use Administer IncuCyte -> Device -> Synchronize as soon as possible.
OK

Figure 8-40. Prompt to Synchronize Time Zone at the Administer Permission Level

Time Zone Synchronization Required
The Time Zone on controller 'IC10094' needs to be synchronized.
Please inform your IncuCyte administrator as soon as possible.
OK

Figure 8-41. Prompt to Synchronize Time Zone at the User or Guest Permission Level

- Navigate to the Administer IncuCyte™ Task Bar and open the Device tab (Figure 8-42). In this example, the local Time Zone is Central Standard Time, but the device (IC10104) Time Zone is Eastern Standard Time. Note that the actual times (~3:28pm) appear to be in agreement despite the fact that the Time Zones have not yet been synchronized. It is VERY IMPORTANT to proceed with the Time Zone synchronization regardless.

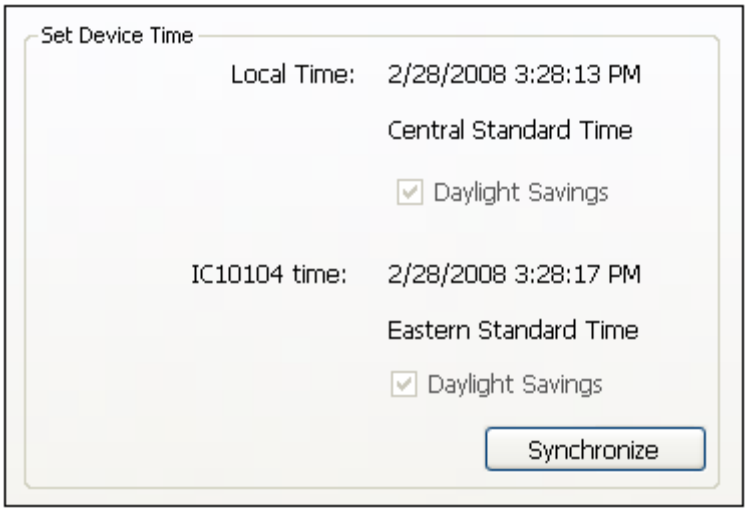


Figure 8-42. Time Zones not Synchronized

IMPORTANT: Even if the local computer and device times appear to be in agreement prior to Time Zone synchronization, it is still ESSENTIAL to proceed with the synchronization process.

Press the Synchronization button to align the disparate time zones. A new window will appear (Figure 8-43). Hit OK to proceed with the synchronization.

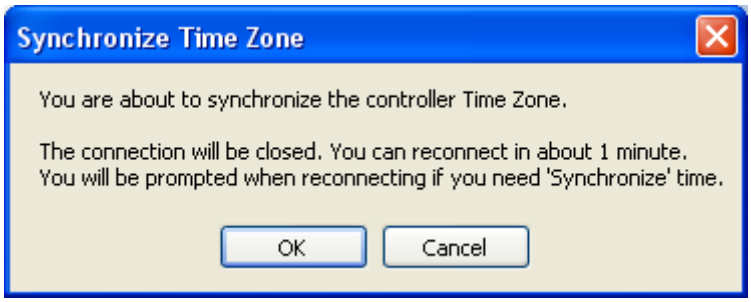

Synchronize Time Zone
You are about to synchronize the controller Time Zone.
The connection will be closed. You can reconnect in about 1 minute.
You will be prompted when reconnecting if you need 'Synchronize' time.
OK
Cancel

Figure 8-43. Synchronize Time Zone

The connection will be terminated, and the device will synchronize the Time Zone. You will be able to reconnect in about one minute, and the IncuCyte™ will be properly synchronized with the local computer.

- **Daylight Savings Time**: Please note that the Daylights Savings Time (DST) check box in the IncuCyte™ software is never active. DST cannot be adjusted through the IncuCyte™ software. It must be selected on your local computer, and the IncuCyte™ settings can be adjusted accordingly.

  Like the Time Zone, DST settings must also be in agreement between the local computer and the IncuCyte™ controller. If this is not the case, a message prompt will appear after login but prior to launching the software. As in the case for synchronization of Time Zones, the exact verbiage of the message will depend on the User's permission level.

  In Figure 8-44 the local computer is not set to DST, but the IncuCyte™ is. In order to synchronize the local and IncuCyte™ controller settings, press the Synchronization button.

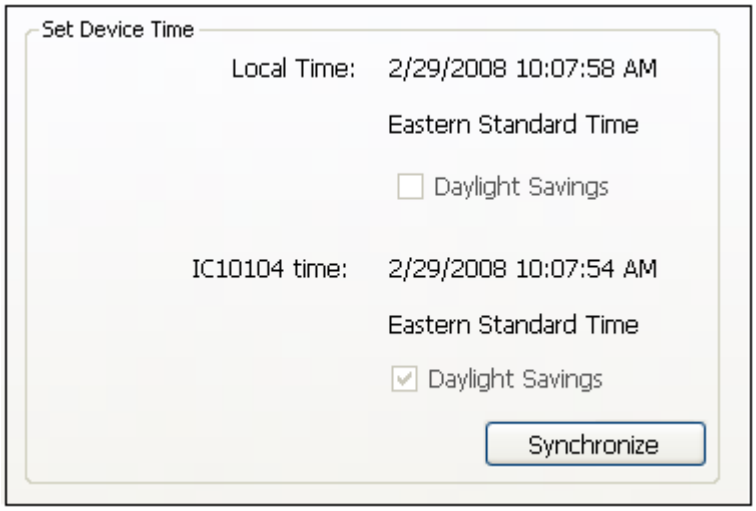


Figure 8-44. Daylight Savings not Synchronized

As in the case for Time Zone synchronization, the connection will be briefly terminated and can be reestablished after about one minute.

Synchronizing the Time

If the difference between the local computer time and the IncuCyte™ time is too great, a message will appear after logging in, but prior to the actual launch of the software (Figure 8-45).

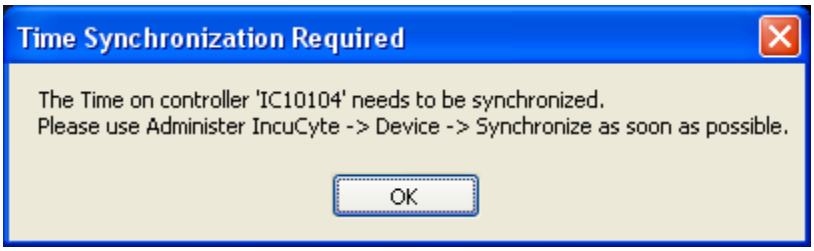

Time Synchronization Required
The Time on controller 'IC10104' needs to be synchronized.
Please use Administer IncuCyte -> Device -> Synchronize as soon as possible.
OK

Figure 8-45. Time not Synchronized

Either proceed to the Administer IncuCyte™ task bar yourself or locate an administrator to synchronize the time. Press the Synchronize button and then press OK in the new window that appears. The times will be is instantly synchronized without leading to any interruption in the connection.

Device System Control

Shutdown

Use the Shutdown button to power down the instrument. This is the recommended method for shutting down IncuCyte™ and is analogous to the Shutdown command on your computer.

Restart

Click on the Restart Device button to reboot the IncuCyte™ controller. Under normal circumstances, the only time the User will restart the device is during the initial installation procedure (according to instructions). However, some IncuCyte™ functions will also cause a restart (such as synchronizing the device time zone/daylight savings).

Export

This is a trouble-shooting function that an Essen BioScience representative will request in the event of a problem. The export will include or more of the following:

- Diagnostics
- Events
- Setup Files
- Export Database

- Tests

Appropriate export instructions will be provided.

8.6.3 Accounts Tab

The next tab in the Administer IncuCyte™ screen is for Accounts. Every IncuCyte™ User must have an account created on each machine in order to access the instrument. Accounts can only be created (and deleted) by an Administrator. A new account can be created for a User at one of three permission levels:

- Guest
- User
- Administrator

For more information on User permission levels, see Section 5.3.2, page 21.

Figure 8-46 shows the Accounts screen as seen at the Administrator level. At the User level, the Accounts screen is the same except that only the Change Password Section is active. There are two major functions available on this screen:

Change Password

To change a password, type the current password and then the new password twice. Click the Change Password button. A window will appear confirming that the password has been changed.

NOTE: The only password that can be changed is the password for the User who is currently logged on. Even an admin level User CANNOT change the password of other Users.

Create User

New Users can be created using the Create User function (Figure 8-47).

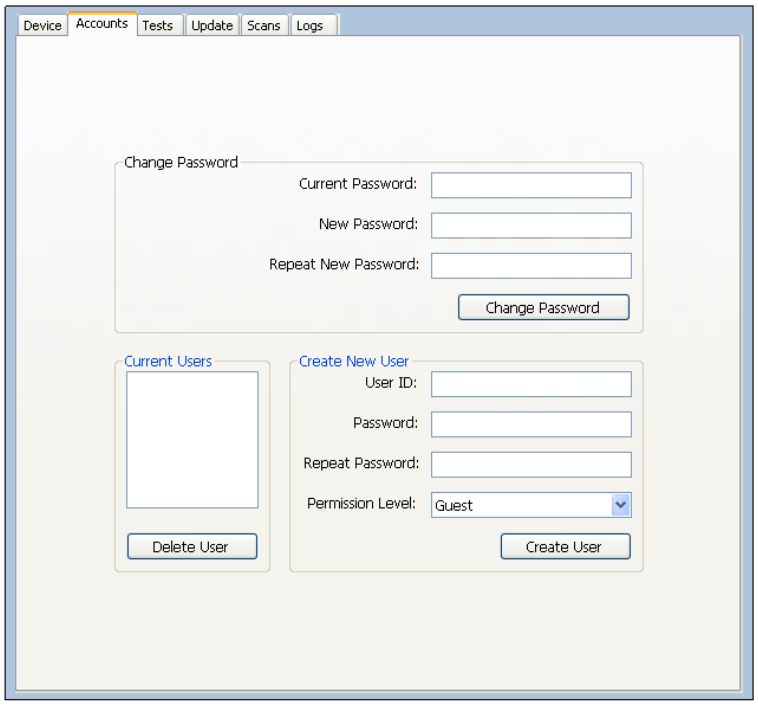


Figure 8-46. Accounts Tab at the Administrator Level

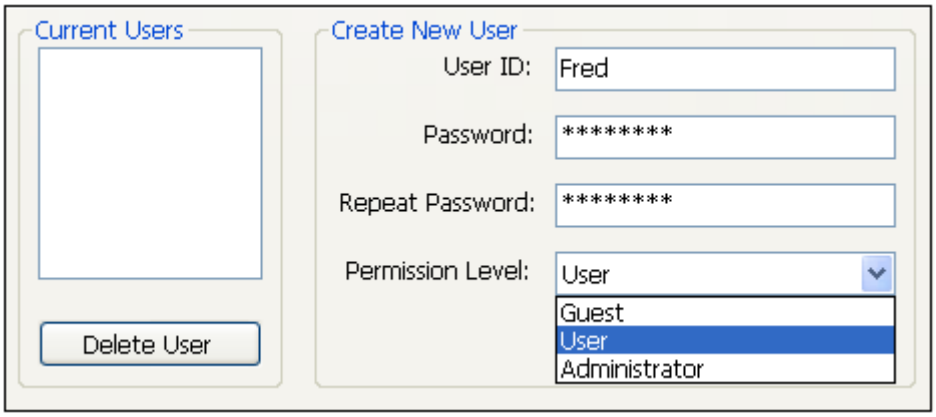


Figure 8-47. Create New User

Create a new User by following these steps:

1. Type the new User's name in the User ID box (in Figure 8-47, the new User's name is Fred).
2. Type in Fred's password once, and then confirm.
3. Use the Permission Level pull-down menu to select Fred's Permission Level (User).
4. Select the Create User button. A window will appear indicating that a new User has been created.

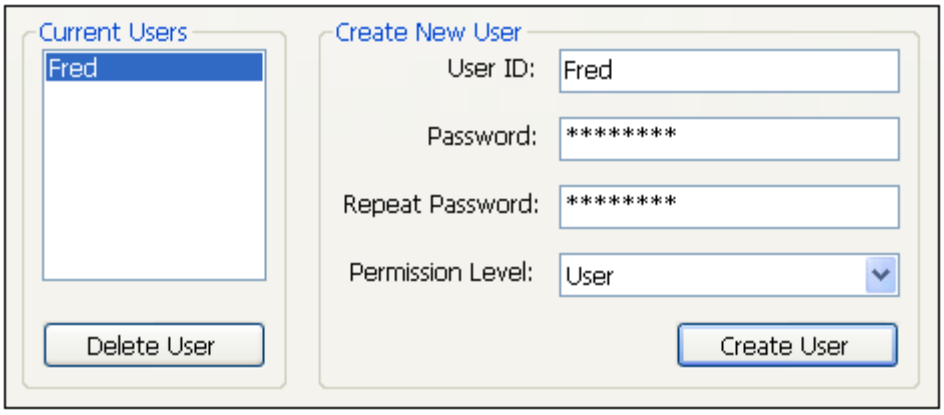


Figure 8-48. New User Created

NOTE: New Users and password changes are effective immediately on creation.

Delete User

Highlight a User name from the Current Users and press Delete User.

NOTE: Unless the Administrator level functions are required, it is recommended that Users log in at the User level because the IncuCyte™ response time will be faster at this level. If administrator level manipulations are required, log out, and then log back on at the Admin level.

EX

8.6.4 Scan Patterns

This tab is ONLY available on the IncuCyte™ EX. To set Scan Patterns on the EX, see Section 8.5.4, page 70.

8.6.5 Tests

NOTE: The Tests tab is ONLY available at the Administrator level.

The Tests tab includes several different tests related to proper IncuCyte™ function. However, the only test that will be relevant to the User under normal circumstances is the Calibration Device test.

Motion Calibration

Before the IncuCyte™ can be used for the first time, the physical locations of the trays must be calibrated within the instrument. A special Calibration Tray is included with the IncuCyte™ for this purpose (Figure 8-49). The calibration tray is the same size and is inserted in the same fashion as the regular trays. Calibration is required before the instrument can be used for the first time in order to establish proper internal alignment. Over time, however, proper

calibration may become somewhat compromised, especially if the instrument is physically moved. If there appear to be any problems with image alignment during scans, it is recommended that calibration be confirmed.

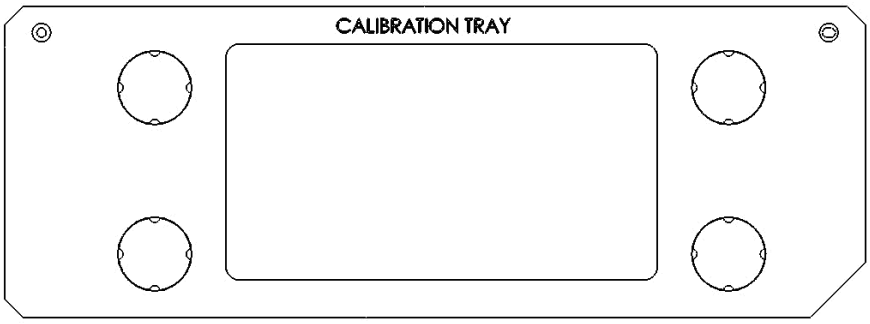


Figure 8-49. Calibration Tray.

To calibrate, complete the following procedure:

Place the Calibration Tray into one of the tray positions (the order in which tray positions are calibrated is not important, but all 3 should be calibrated). For the example in Figure 8-50, start with Tray Position: Front. The Front, Middle and Rear tray positions can be selected using the pull-down menu. Each Tray Position must be individually calibrated and/or confirmed.

Select Test: There are 3 test options to choose from.

- **Calibrate and Confirm:** Run this test the first time the instrument is calibrated. Press Run. A window will appear to confirm that the Calibration Tray has been loaded into the appropriate position. Select Yes and a new window will appear confirming that calibration has begun (press OK). The IncuCyte™ will calibrate the tray in the designated position and follow up with a test to confirm that the calibration is correct. If the calibration tray is absent or was not placed into the appropriate tray, the process will abort.

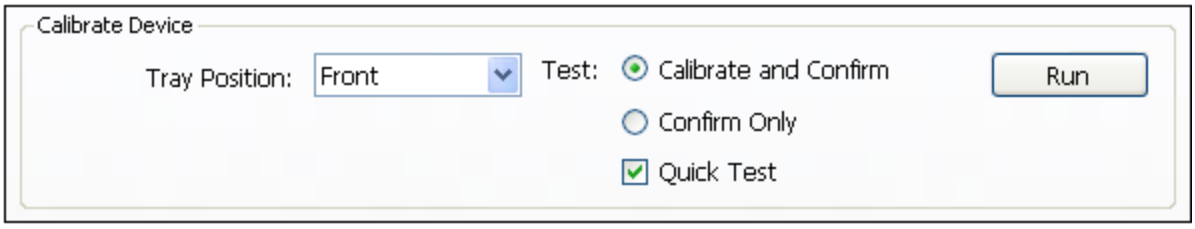


Figure 8-50. Motion Calibration with Calibrate and Confirm and Quick Test selected for the Front Tray Position.

- **Confirm Only**: If the instrument has been previously calibrated, but there is some question as to whether the calibration is still accurate, calibration can be confirmed using Confirm Only. This single test requires less time than the Calibration and Confirm tests together, and it is useful for obtaining a rapid update on calibration status.

- **Quick Test:** In most cases, the Quick Test will be used for both the Calibrate and Confirm or just the Confirm tests. The Quick Test is not a separate kind of test, but refers to the length/detail with which the Calibrate/Confirm tests are performed. The Quick Test check box should be checked along with the selected test type (Figure 8-50). If the Quick Test box is not checked, the Calibration/Confirm tests will require significantly more time. If the Quick Test fails, repeating the test with the Quick Test check box unchecked will not increase the chances that the test will succeed. However, it will provide more information that can be used to trouble-shoot the source of the problem. Generally, if the Quick Test fails, simply try running the test again. If the test fails repeatedly, then contact Essen BioScience.

The Results of the Calibration and/or Confirm tests can be viewed under the Logs tab. Select Logs, and then use the pull-down menu under Log Type to select Diagnostic. The calibration results will also be visible under the Log Type category All. The results will be displayed in the large window below. If no results are visible, press the Refresh button at the bottom of the screen to update the log. The results will be displayed as coordinate values. These values will not be important to the User under normal circumstances. At the end of the coordinate tables, the Log will indicate whether the test Passed or Failed (Figure 8-51).

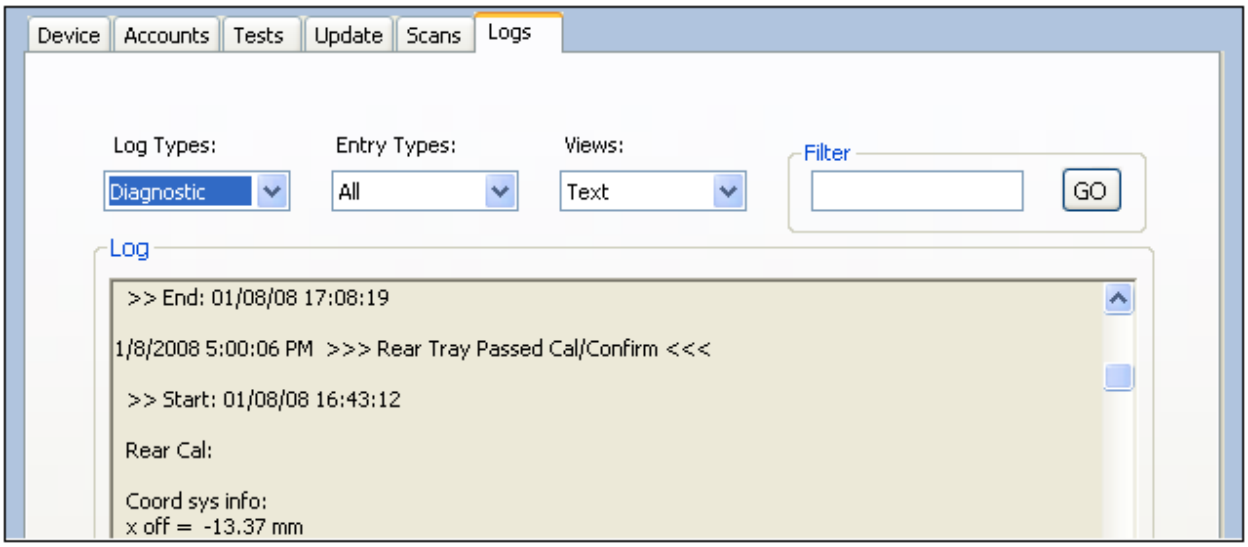


Figure 8-51. View the calibration results under the Logs tab, Log type Diagnostic

All three tray positions must be individually calibrated. When one tray position has completed the test(s), move the Calibration Tray to the next position and repeat. When all positions have been calibrated, the instrument will be ready to use.

If the results of the calibration are not displayed, press the Refresh button at the bottom of the screen to bring all the logs up to date.

If the calibration was performed recently, the results will appear at the top of the log window. However, if the object is to locate calibration results from some point in the distant past, they will likely be preceded at the top by other, more recent events The number of entries displayed at any given point can be adjusted at the bottom of the Logs tab using the Entries to Display pull-down menu. Try increasing the number of displayed entries if you are unable to locate your results within the current log screen.

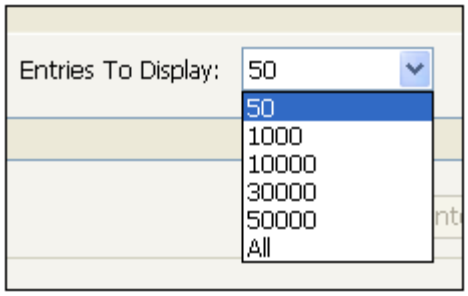


Figure 8-52. Select the number of entries to be displayed in the Log window.

HD

384-Well Plate Calibration (HD only)

The standard calibration procedure previously described in Section 6.2 is adequate for scanning all vessels in the IncuCyte™ HD with the important exception of 384-well plates. Because scanning of 384-well plates requires such a high degree of precision, it also requires a special calibration procedure. Again, all three IncuCyte™ tray positions (front, middle, rear) must be individually calibrated for 384-well plates.

In order to calibrate the IncuCyte™ for 384-well plate scanning, complete the standard calibration procedure as described in Section 6.2. The additional 384-well Plate Calibration requires a single microplate tray and two (2) EMPTY clear-bottom, opaque-walled, 384-well plates. The following is a list of 384-well plates that have been tested and approved for 384-well Plate calibration use:

Manufacturer:	Wall Color:	Catalogue Number:
BD Biosciences	Black	353962
Corning	Black	3712
Greiner	Black	781091
Greiner-GNF	Black	789070-G
Matrix	Black	4332
	White	4330
Nunc	Black	142761
Porvair	Black	312003
	White	311003

Figure 8-53. 384-Well Plates Approved for HD Calibration

We recommend that customers select the same plate type for calibration that they also plan to use most frequently for scanning. If the desired plate type is not among our approved list of candidates, then select the plate type that seems closest to your particular plate of interest. Clear-walled plates CANNOT be used for 384-well Plate calibration, so in that case select the approved plate from the same manufacturer.

It is helpful to pre-warm plates prior to using them for calibration. If room temperature plates are placed directly into the warm incubator, condensation can form on the bottom of the plates and interfere with the calibration results.

ONLY clear bottom, COMPLETELY EMPTY, opaque-walled 384-well plates from the approved list can be used for the 384-well Plate calibration procedure.

Follow these steps to for the 384-well Plate calibration procedure (Figure 8-54):

Put two (2) empty 384-well plates into a microplate tray (both spaces in the microplate tray must be occupied).

Select the appropriate tray position in the software – all three tray positions need to be calibrated.

Press Run. The calibration for each tray position takes only a few minutes.

Repeat for all three tray positions.

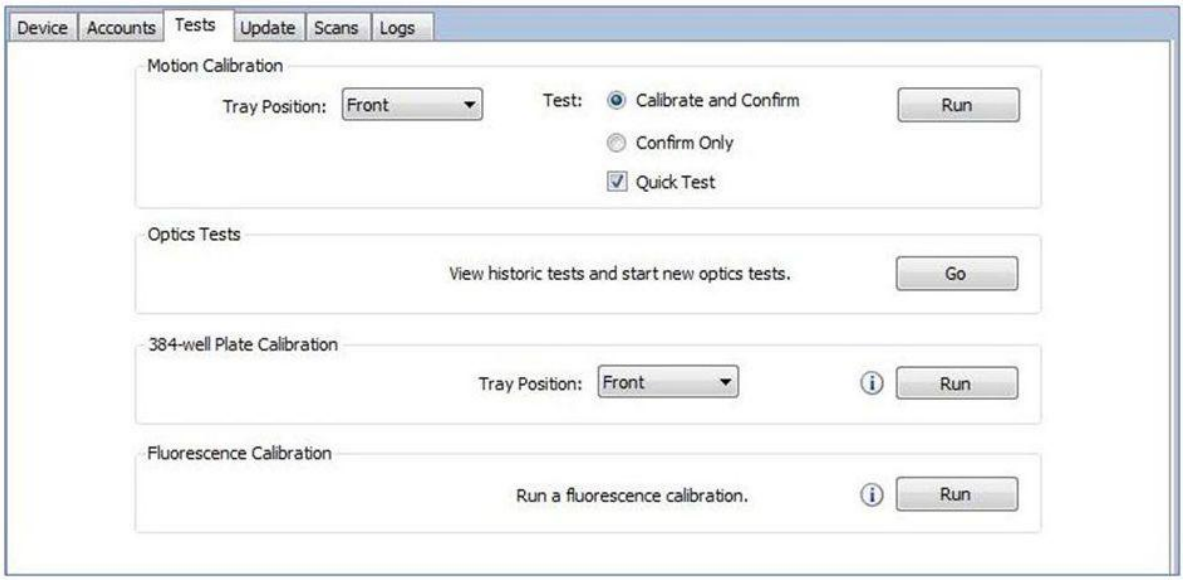


Figure 8-54. 384-well Plate Calibration

The results for the calibration can be viewed under the Logs tab, Log Type Diagnostic. Unless there is an error message in the log file, the calibration was successful.

EX

Calibrate EX

The IncuCyte™ EX does not require a special calibration tray. The built-in vessel tray also serves as the calibration tray. To calibrate the EX, select the Tests tab under the Administer IncuCyte™ task bar (Figure 8-55). Calibration is identical to that of the Standard Model IncuCyte™ except that the Tray Position cannot be selected (because the EX contains only a single tray). View the calibration results in the Logs tab under Log Type: Diagnostic.

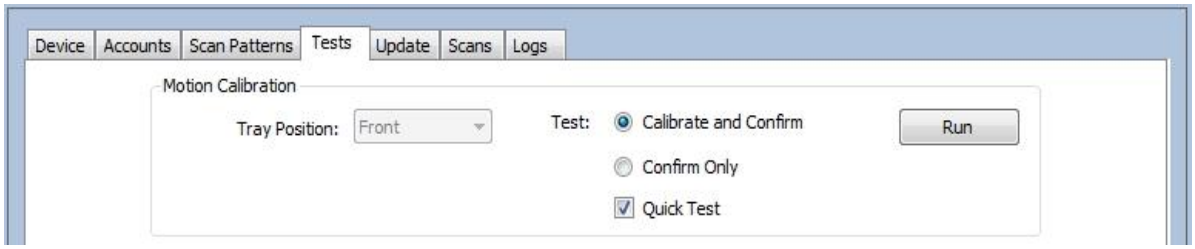


Figure 8-55. EX Calibration Screen

FLR

Fluorescence Calibration (FLR only)

Because all IncuCyte™ FLR's are HD-enabled instruments, they must undergo standard tray calibration as well as HD calibration. Additionally, the fluorescent IncuCyte™ requires a special fluorescence calibration procedure. See the Essen BioScience IncuCyte™ FLR Application Note for more information.

Tech Note

A calibration kit will be provided with the purchase of the IncuCyte™ FLR, and the instrument will be calibrated at the time of installation. It is currently recommended that the instrument be recalibrated approximately twice a year; however, if the User suspects a drift in the calibration signal, then the system can re-calibrated at any time.

The fluorescence calibration kit includes liquid sufficient for multiple calibrations as well as three calibration slides. These calibration slides are disposable and should only be used once. New calibration kits can be purchased from Essen BioScience. See Section 13, IncuCyte™ Catalog, page 244.

Follow these steps to perform the fluorescence calibration:

Place a microslide tray in the front position of the IncuCyte™. Fluorescence calibration requires use of the front tray position, and the remaining positions can be occupied with trays at the time of calibration.

Load a calibration slide with supplied dye. Use 40µl dye per slide. Place the pipet tip at one end of the slide window and expel the liquid dye. The liquid will wick into the slide window. After all the dye has been expelled, use the pipet to transfer a small amount of dye to the other end of the window to be sure it is completely filled. Avoid getting any dry spots in the window as this will interfere with proper calibration.

Place the calibration slide in the FAR LEFT CUTOUT POSITION of the microslide tray. The orientation of the slide within the cutout position is not important. However, it is ESSENTIAL that the calibration slide is the ONLY slide in the microslide tray. Verify that the rest of the tray is empty.

Select the Tests tab under the Administer IncuCyte™ tasks bar. Then, locate the Fluorescence Calibration test and press Run. A new window will open to confirm that the IncuCyte™ is properly configured to run the test. Press the Yes button to start the calibration (Figure 8-56).

The calibration procedure will begin. Fluorescence Calibration requires ~ 15 minutes. Calibration status will be indicated at the top of the IncuCyte™ software window and in the status box at the bottom left.

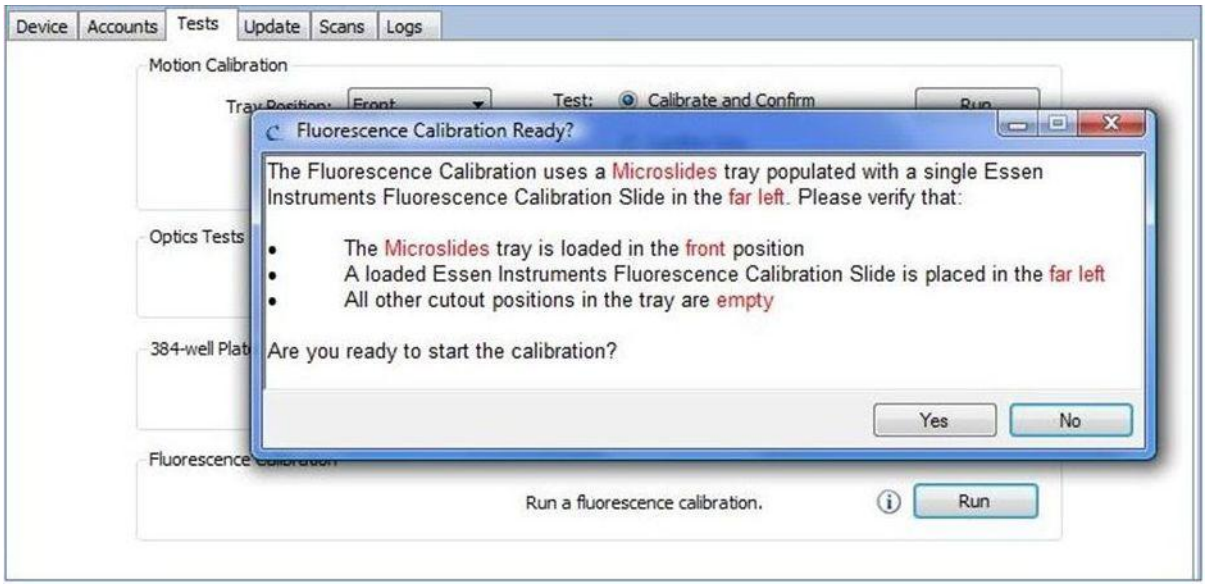

Device
Accounts
Tests
Update
Scans
Logs
Motion Calibration
Test:
Calibrate and Confirm
Fluorescence Calibration Ready?
The Fluorescence Calibration uses a Microslides tray populated with a single Essen Instruments Fluorescence Calibration Slide in the far left. Please verify that:
The Microslides tray is loaded in the front position
A loaded Essen Instruments Fluorescence Calibration Slide is placed in the far left
All other cutout positions in the tray are empty
Are you ready to start the calibration?
Yes
No
Optics Tests
384-well Plat
Run a fluorescence calibration.
Run

Figure 8-56. Fluorescence Calibration

Calibration Results

When calibration is complete, the results can be viewed under the Logs tab, Log Type: Diagnostic. The log will state whether or not the calibration passed. If the calibration failed, the log will include recommendations to avoid failure the next time through.

Optics Test

The Optics Test is a trouble-shooting function that would be requested by an Essen BioScience employee. It tests the quality of the camera image. In order to run the Optics Test, the front position of the IncuCyte™ drawer MUST be COMPLETELY empty (no tray).

Running the Optics Test

To run an optics test, follow the steps indicated below:

Press the Go button in the Optics Test Section. This will open the Optics Test window (Figure 8-57). The dates and times of previously run optics tests will appear in the top, left portion of the window. The image result for the highlighted/selected test will be displayed to the right of the dates and times, and the written results for the highlighted test will be displayed below the image. If no optics tests have been performed on the instrument, the Current Test image will contain the words No Image Found.

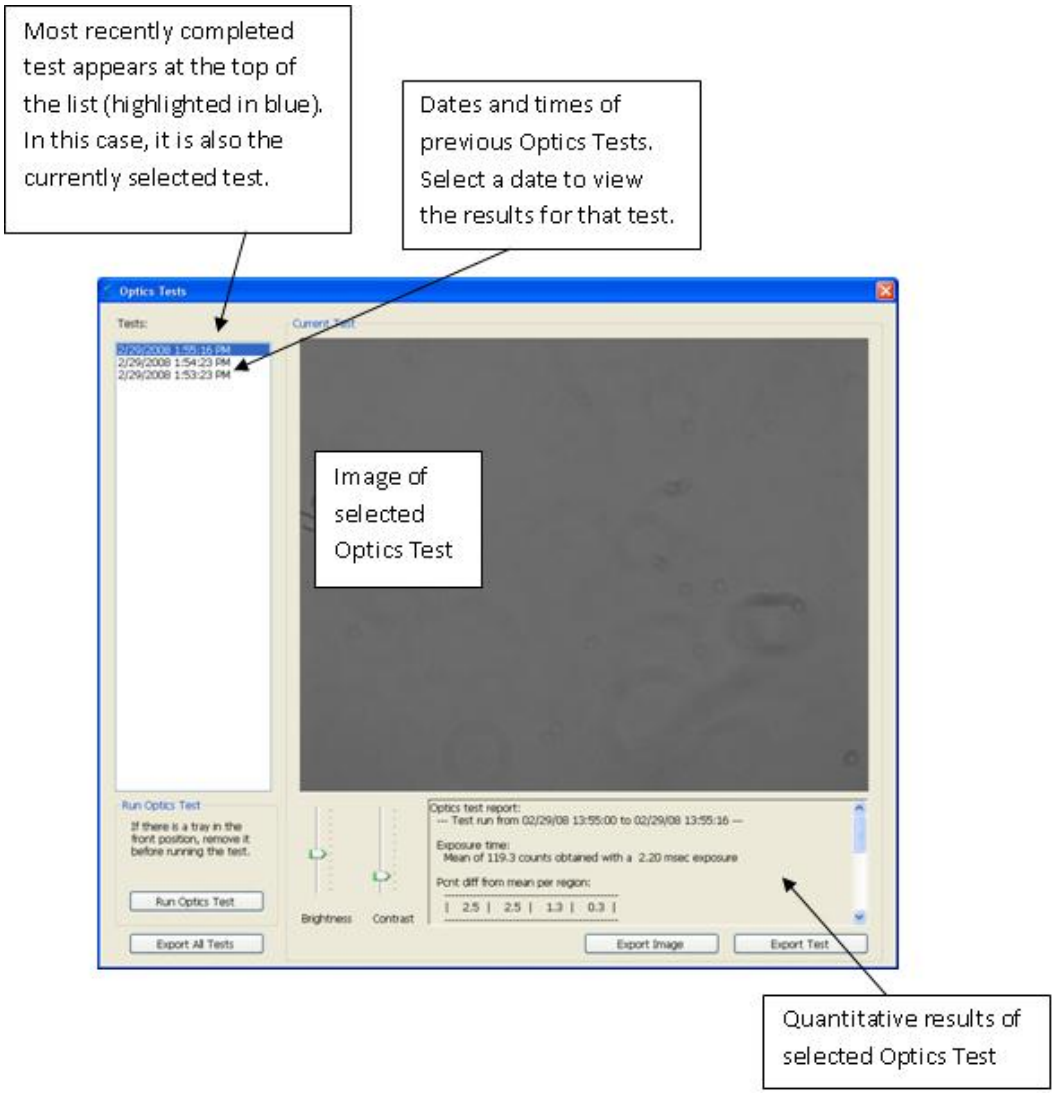

Most recently completed test appears at the top of the list (highlighted in blue). In this case, it is also the currently selected test.
Dates and times of previous Optics Tests. Select a date to view the results for that test.
Optics Tests
Tests:
Current Test
2/29/2008 1:55:16 PM
2/29/2008 1:54:23 PM
2/29/2008 1:53:23 PM
Image of selected Optics Test
Run Optics Test
If there is a tray in the front position, remove it before running the test.
Run Optics Test
Export All Tests
Brightness
Contrast
Optics test report:
Exposure time:
Mean of 119.3 counts obtained with a 2.20 msec exposure
Pcnt diff from mean per region:
2.5 | 2.5 | 1.3 | 0.3
Export Image
Export Test
Quantitative results of selected Optics Test

Figure 8-57. The Optics Test Window

Initiate a new test by pressing the Run Optics Test button

- A window will appear to indicate that the front position of the IncuCyte™ must be empty for the test to function appropriately. If this position is empty, then press Proceed. If not, press Cancel, remove any tray in the front position, and then start again.
- A new window (Optics Test Started) will appear indicating that the optics test has been initiated. The Optics Test window will become inactive while the test is running. When the test is complete, the Optics Test window will become active once again, and the time and date of the most recently completed test will appear above those of previous tests. The Optics Test Started window will remain open until it is closed by pressing OK.

Exporting Results

Three options are available to export the optics test results:

1. Export Image
2. Export Test
3. Export all Tests

An Essen BioScience employee will indicate which test(s) need to be exported. Pressing any of the Export buttons will open a Save As window to select a location for saving the exported file. Save the file to the appropriate location and then email it to Essen BioScience as requested.

EX

Optics Test EX

The Optics Test for the IncuCyte™ EX can be run as described for the Standard Model Optics Test with one exception: because the EX has a single, built-in tray that cannot be removed, run the test with the tray empty (i.e. no vessel in the tray).

Camera Test

The Camera Test is another trouble-shooting function that would be requested by an Essen BioScience employee. To run a camera test, press the Run button in the Camera Test box. A window will appear to confirm that the camera test has begun. Hit OK. The status of the camera test will be indicated at the top of the IncuCyte™ screen. In Figure 8-58 the camera test is 4% complete.

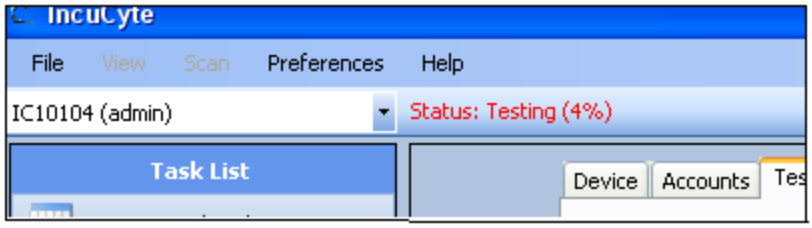


Figure 8-58. Camera Test Status

The results of the camera test can be viewed under the Logs tab, in Log Types: Diagnostic. (See Section 8.6.8: Diagnostic, page 121).

8.6.6 Update

NOTES:

The Update tab is ONLY available at the Administrator level.

New Licenses are issued on a per instrument (controller) basis. Therefore, if a new license is purchased, it can only be used on the instrument onto which it was installed.

From time to time, various updates will become available to improve or expand upon IncuCyte™ function. In order to perform any of these updates, Essen BioScience will dispatch an email containing the attached update file(s) and the corresponding installation instructions. The three different update options are described below.

Update Database

Your IncuCyte™ will arrive equipped with the most recently available database of compatible cell culture vessel types (see Section 6.1, Selecting and Placing Trays and Vessels into the IncuCyte™, page 25) and a selection of trays to accommodate them. However, as new vessel types are created, and as customer demand requires, additional vessel types and trays will be added to the database. Any such updates can be uploaded into your IncuCyte™ using the Update Database button.

Load License

New applications for the IncuCyte™ are being developed as an ongoing process. Some specific applications will require additional software modules in combination with the standard software package. To upload these new software applications, select the Load License button.

IMPORTANT! Before loading a new license or upgrading an IncuCyte™, it is ESSENTIAL to remove any vessels from the Schedule Upcoming Scans screen and then press Apply (it's ok if the vessels/trays are still physically located in the IncuCyte™). If any vessels are present (even if no scans are scheduled) when the new license is loaded, the software will experience a serious problem. Therefore, completely clear the Schedule Upcoming Scans screen before loading a new license.

Update Device

As new versions of the IncuCyte™ software become available, they can be uploaded using the Update IncuCyte™ button.

8.6.7 Scans Tab and Archiving for Data Management

NOTE: The Scans tab is ONLY available at the Administrator level; however, it is possible to archive individual vessels at the User and Guest Levels. See Archive Vessels, page 114.

Device Status

When scans are initially carried out, the data are stored on the IncuCyte™ controller. While the controller can hold a large amount of data, it will gradually fill up as more and more scans are performed. The amount of free space remaining on the controller hard drive is displayed at the bottom left of the Schedule Upcoming Scans screen in the Device Status box. Figure 8-59 displays the Device Status box, which indicates that 445.43GB (97%) of free space

remain on the controller hard drive. In order to free up space on the controller hard drive, scan data can be removed from the controller to a new destination through a process called archiving.

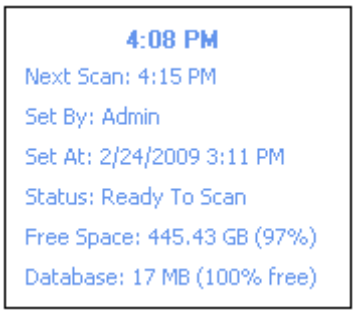


Figure 8-59. The Device Status Box

FLR

IncuCyte™ FLR Temperature History

As a quality control measure and diagnostic tool, IncuCyte™ FLR is equipped with a temperature sensor and graphing tool for viewing the temperature history inside an IncuCyte™. A current temperature reading for the instrument is available at the bottom of the Device Status box (Figure 8-60). To access your instrument's temperature history, click on the graph box next to the temperature reading to bring up the temperature history window (Figure 8-61). Here, scan periods are shown in red, while idle periods are blue. At the bottom right of the screen, choose to view the last 24 hours, one week, or 30 days. As with the standard graphing window, click and drag over an area within the graph to zoom in on that area.

The IncuCyte™ FLR temperature sensor makes periodic measurements of the ambient temperature inside the instrument. Although this sensor is very accurate, it only approximates the internal temperature of the device due, as it is in a fixed location and does not move around with the microscope. Nonetheless, the sensor is very useful for measuring the heating and cooling trends in the device over long periods of time. By plotting the temperature over time, one can not only track the rise and fall of the temperature as the device scans, but also as the heat that is both lost from the incubator (by opening the door) and injected into it by the incubator heating system. The temperature sensor, therefore, is a useful tool for verifying the target temperature setting of the incubator itself as well as for tracking the influence of IncuCyte scans on the transient temperature inside the device. See Scan Length, for recommendations on how to minimize the influence of the IncuCyte™ on incubator temperature.

Figure 8-60. The Device Status box in IncuCyte™ FLR.

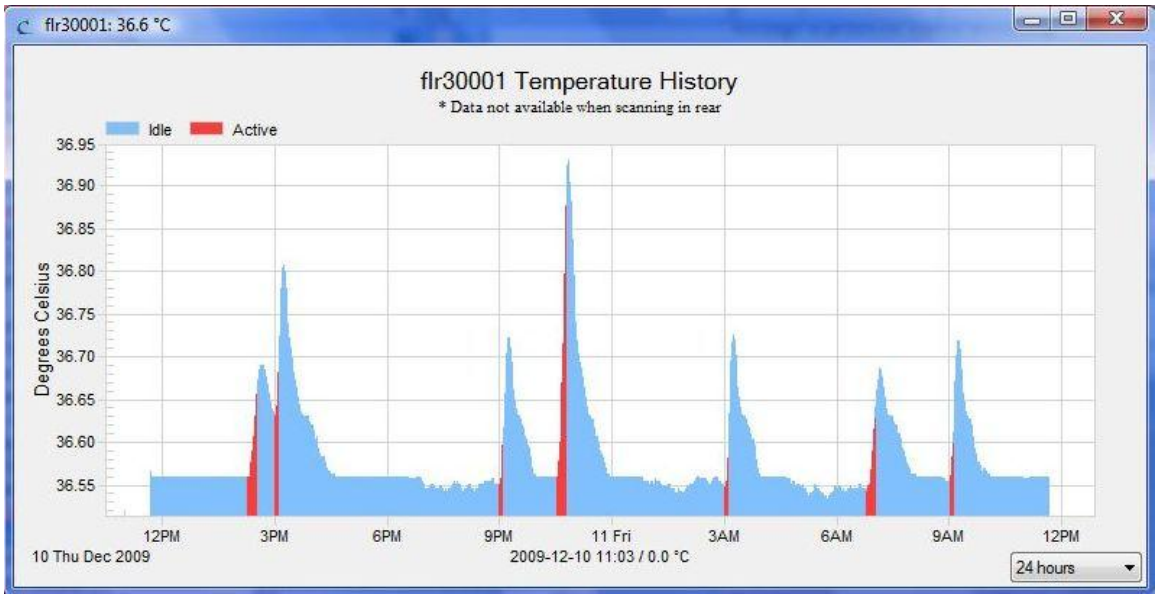


Figure 8-61. The Temperature History window.

The Three Basic Scans Tab Functions

Use the Scans tab screen (Figure 8-62) to archive data. There are 3 related functions available on this screen:

- Archive Scans
- Delete Scans
- Restore from Archive

The desired Task can be selected using the Select a Task pull-down menu.

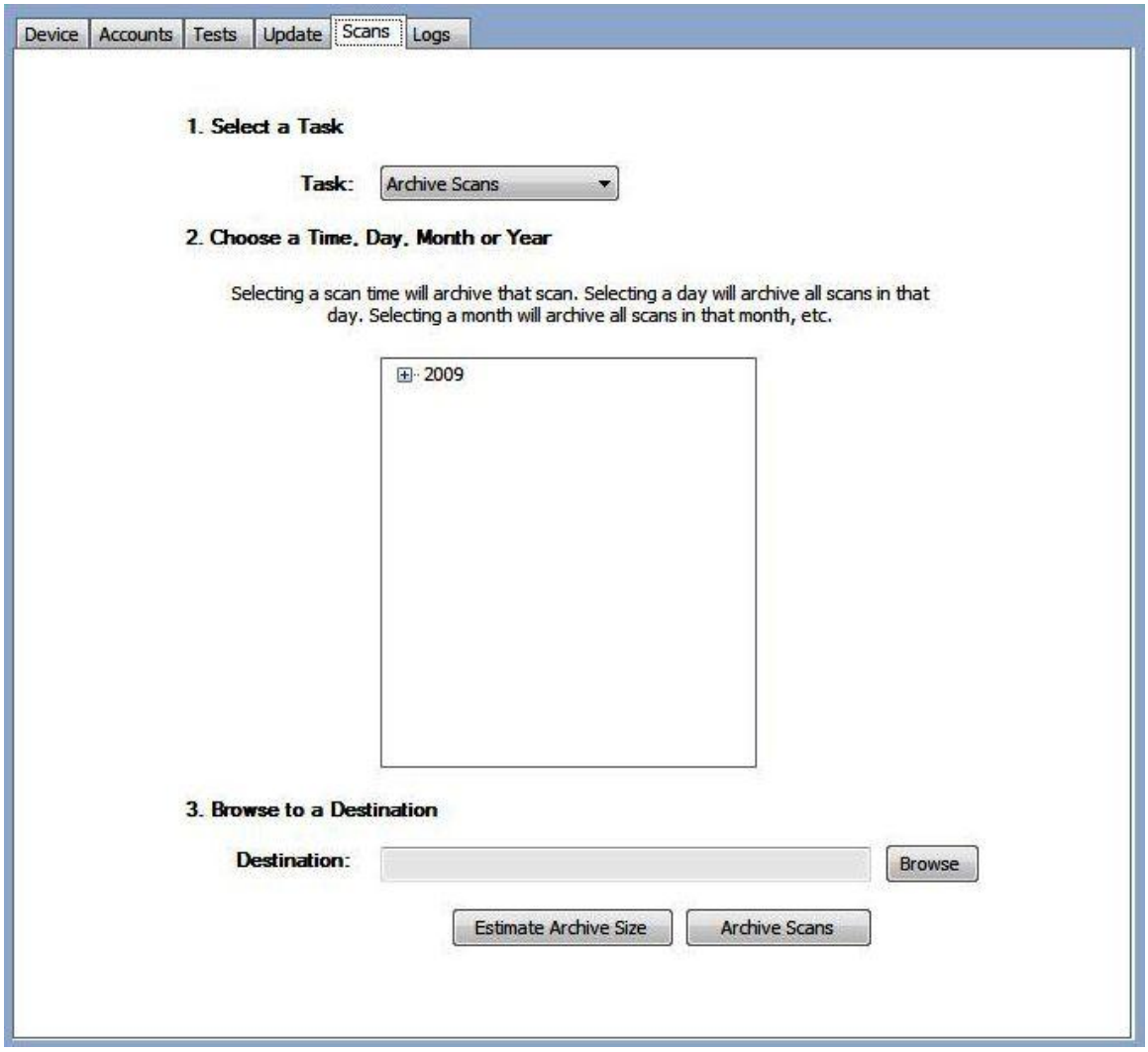


Figure 8-62. The Scans Tab

Archive Scans for Data Management

The IncuCyte™ controller hard drive has limited space and may require a data management plan to archive and delete information from the system. This plan may vary depending on usage and number of users. The recommended best practice is to archive individual experiments once completed (see Archive Vessels). After the scans have been archived a user with administrative permissions can then delete the experiment in its entirety. If this recommendation is not feasible, archives can be made on a weekly or monthly basis. It is not recommended to archive more than three months' worth of data to a file share across a network. Due to network limitations and traffic the archive may timeout resulting in an incomplete file copy.

Archive Scans

Use the Archive Scans task to move scan data from the IncuCyte™ controller hard drive to a new destination. All scans available for archiving are displayed in the Time Tree in the Choose a Time box. To select a single time for archiving, highlight that time with the mouse. To select a series of scan dates/times for archiving, highlight the first desired scan time with the mouse, hold down the Shift key, and then select the final desired scan time. All scans including and between the first and last selected scan times will be highlighted for archiving. To select several scan times for archiving that do not occur in a series, highlight the first desired scan time, hold down the Ctrl key on the keyboard, and then select all remaining desired times. As indicated in the Scans Screen, selecting a day, month, year, etc. will archive all scans within that selected timeframe. In Figure 8-63 the scans for Saturday, January 12th 2008, 2:19am through January 12th 2008, 11:19pm have been selected for archiving.

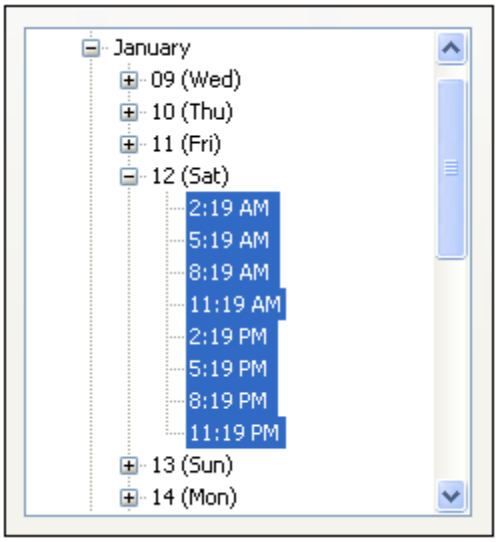


Figure 8-63. A series of scans is selected using the Shift Key.

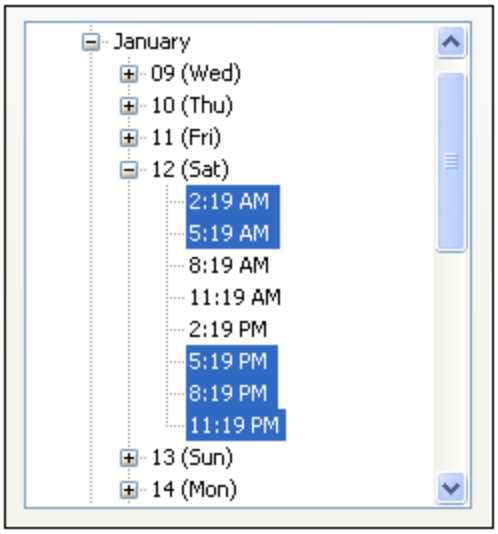


Figure 8-64. A collection of scans is selected using the Ctrl key.

After scan times have been selected, use the Browse to a Destination box to select the new location for the archived data. Select the Browse button. Choose the desired location, and then click Save. The new destination will now be displayed in the Browse to a Destination box (Figure 8-65).

Note: Select the Estimate Archive Size button to display the approximate file size of the archive file on the disk.

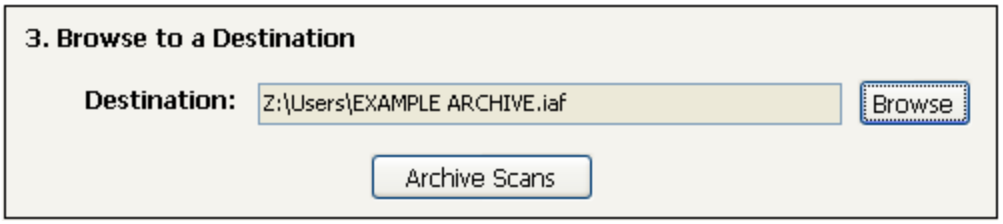


Figure 8-65. Browse to a Destination

Now, select the Archive Scans button. A window will appear to confirm that archiving is desired. Once the archiving process has been initiated, it cannot be interrupted. Press OK. Another window will appear that displays archive status. Archiving can require a significant amount of time if large numbers of scans are being archived.

After archiving is complete a new Archiving Complete window will appear. Any problems encountered during the archiving process will be displayed in this window. In Figure 8-66 no errors were encountered during archiving.

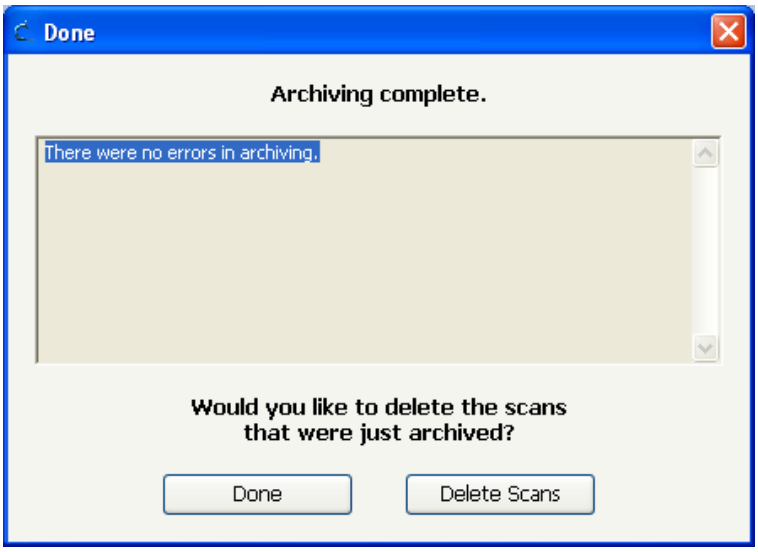


Figure 8-66. Archiving Results

Note that the archiving process copies the selected scan data from the controller to its new location. Therefore, after the process is completed, these data still remain on the controller hard drive. Generally, it is a good idea to delete these data from the controller following archiving in order to free space on the hard drive. If the controller hard drive is full, new scans CANNOT be performed.

The Archiving Complete window conveniently presents the opportunity for deleting the previously archived scans. However, ONLY these scans can be deleted at this point. To delete other scans, see the Delete Scans Section that follows. If deletion is not desired at this time, simply click Done, and the entire process will be complete (with no scan deletion). However, if the archived scans should be deleted, click on Delete Scans. These Scans will be PERMANENTLY deleted. Once scans have been deleted from the controller, they CANNOT be retrieved. Therefore, consider carefully before deleting any scans.

Note: Multiple archives CANNOT be saved to the same folder. A folder must be created for each individual archive.

Delete Scans

The second option under the Task pull-down menu is Delete Scans (Figure 8-67). This task can be used to delete scan data from the controller hard drive. Unlike the Archive Scans option, selected scans will NOT be copied prior to deletion. If the controller hard drive is full, new scans CANNOT be performed. Scans are selected for deletion using the Time Tree in the same way as they are selected for archiving (see Section 8.6.7, Scans Tab, page 110).

After scans have been selected, simply press the Delete Scans button. A window will appear to confirm that scans should be deleted.

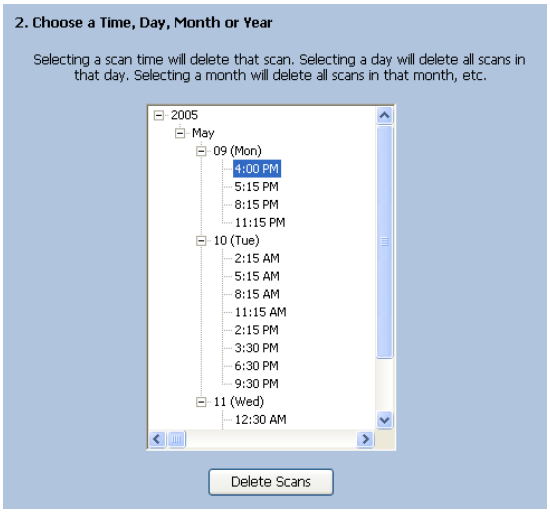


Figure 8-67. Delete Scans.

Restore from Archive

Under some conditions, scans that were previously archived can be restored from their archive location back onto the IncuCyte™ controller. After scans have been restored, they appear on the controller as they did prior to archiving, and they do not need to be opened separately by browsing to an archive. This function is available with some limitations:

- The Restore Archive Function is only available on IncuCytes™ equipped with a 2009A or later software release.
- Archived scans can only be restored if they were performed and archived using an IncuCyte™ equipped with the 2009A or later software release.
- The archive must originate from the same controller to which it is being restored.

Restoring archives is only recommended under 2 circumstances:

- Searching Vessels (archives cannot be searched)
- Running Analysis Jobs. See Manipulation of Archived Data FLR, page 117.

To restore an archive browse to the archive location and then select the scans to be restored.

Archive Vessels

When scans are archived as described in Section 8.6.7 (page 110), all vessels on the screen will be archived together into a single archive file. However, it is

also possible to archive one or more individual vessels while not archiving others from the same screen. There are 3 ways to archive vessels:

- Select Archive Scans from the View Completed Scans Scan pull-down menu (Figure 8-68).
- Right-click on the desired vessel in the View Completed Scans screen.
- Access Archive Current Vessel from the Vessel View Utilities pull-down menu or in the Vessel View Tasks Pane.

Unlike entire scan archives, it is NOT possible to append to a vessel archive.

NOTE: The Scans tab is only available at the Administrator level; however, individual vessels can be archived at the User and Guest levels.

The remaining functions available under the Scans pull-down menu will be discussed in Section 8.7.9 (page 152).

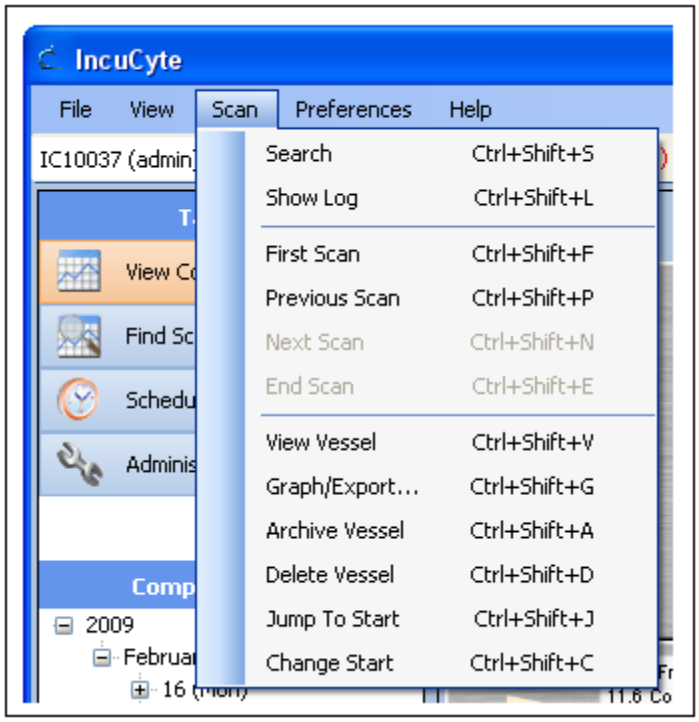


Figure 8-68. The Scans Pull-Down Menu

Selecting Archive Vessel will open the Archive Vessel window (Figure 8-69). Select the Browse button to choose the archive destination. Then, select Archive Vessel to initiate the archiving process. To see the size of the archive, select Estimate Archive Size.

As in the case for archiving scans, use the Shift key to select a series of scans for archiving, or use the Ctrl key to select a collection of scans for archiving.

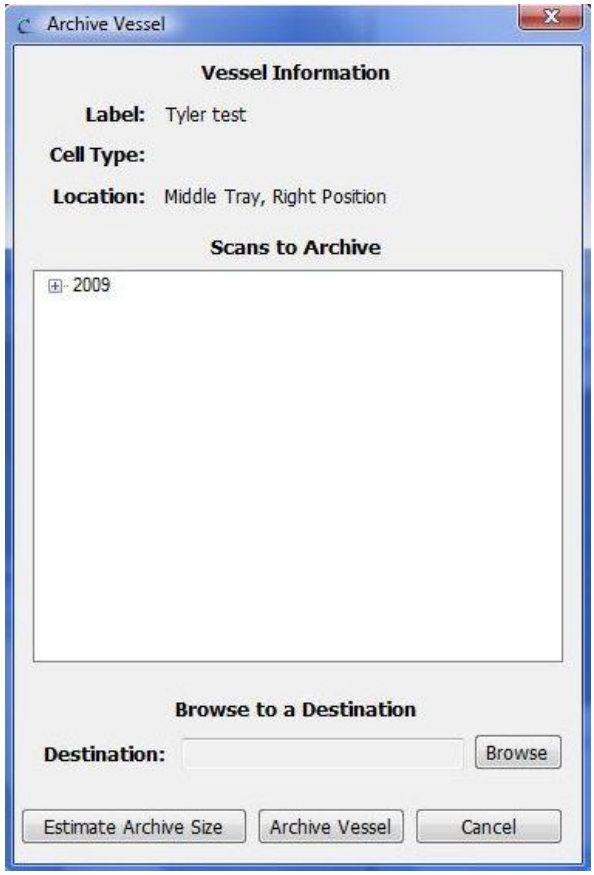


Figure 8-69. Archive Vessel Window

Delete Vessels

Individual vessels can be deleted without having to delete an entire scan. The Delete Vessel function can be accessed by:

- Selecting the Delete Vessel in the Scans pull-down menu (Figure 8-68).
- Right-clicking on the selected vessel in the View Completed Scans screen. See Tasks Available by Right-Clicking on a Selected Vessel, page 131.

Once a vessel is deleted, it can only be restored from a vessel archive made with 2009A or later software.

Additional Archive Notes

Appending to an archive

When multiple archives are generated, they can be either created in new files or added to existing archive files. To add additional archive data to an existing file,

simply browse to the desired, existing .iaf destination file (when selecting the archive destination) and select Save. A window will appear indicating that the selected file will be Appended To. Appending a file will NOT result in any previously existing data being overwritten.

It is NOT possible to append to a vessel archive.

Manipulation of Archived Data

Archived scans can be opened and manipulated to the same extent as scans saved on the controller. However, manipulations of archived data will be slower than that of data remaining on the controller hard drive - especially if the archive is large. Thus, while regular archiving is essential for long-term IncuCyte™ use, it is recommended that scans remain on the controller as long they are being actively manipulated. To open archived data see Open an Archive under Section 8.6.7 (page 117).

FLR

Manipulation of Archived Data FLR

Fluorescent scans can be archived and manipulated, but it is NOT possible to run Analysis Jobs from archives. In order to run an Analysis Job from scans that have been archived, the scans must first be restored using the Restore Archive function. See Restore from Archive (page 114). See Section 9.3 (page 204) for information regarding FLR-specific applications and analysis.

Although analysis jobs cannot be run from archived scans, any analysis jobs that were completed prior to archiving will still be available after the vessel/scan has been archived. Thus, it is possible to archive analysis jobs as well as vessels.

Default Name

Archived data will always be given the default name Archive.

Open an Archive

Archives can be opened the same way as opening an IncuCyte™ with a User account. Simply click on the IncuCyte™ icon or select Open a Connection under the File pull-down menu. When the Open Connection window appears, select Browse to an Archive and locate the desired archive file (Figure 8-70). Unlike opening a connection with an IncuCyte™, a password is NOT required to open an archive.

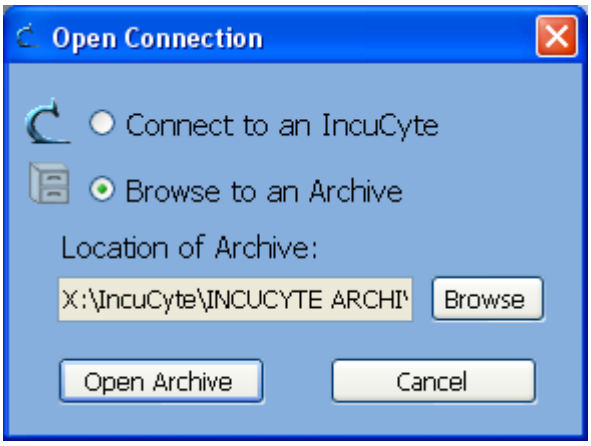


Figure 8-70. The Open Connection Box

As mentioned previously, when an archive is saved, both a folder and a file will appear in the target location. Both will be assigned the default name, archive; although the file will bear the name archive.iaf (IncuCyte™ Archive File). To select the archive from the Browse location, double left-click on the .iaf file (Figure 8-71).

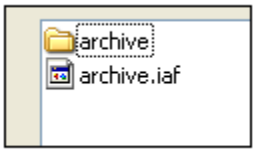


Figure 8-71. Open Archive. To open the archive, click on the archive.iaf file.

The path to the archived data will then be displayed in the Browse window. Select the Browse button, and IncuCyte™ will open the screen for the desired archive (Figure 8-70). In the Archive Screen, the path name to the archive file is listed at the top, left corner of the screen, and only the View Completed Scans task list bar is available. No scans can be set and no administrative functions can be performed from an archived data screen.

All IncuCyte™ features that are available (under the View Completed Scans screen) outside of an archived screen are also available from archived data.

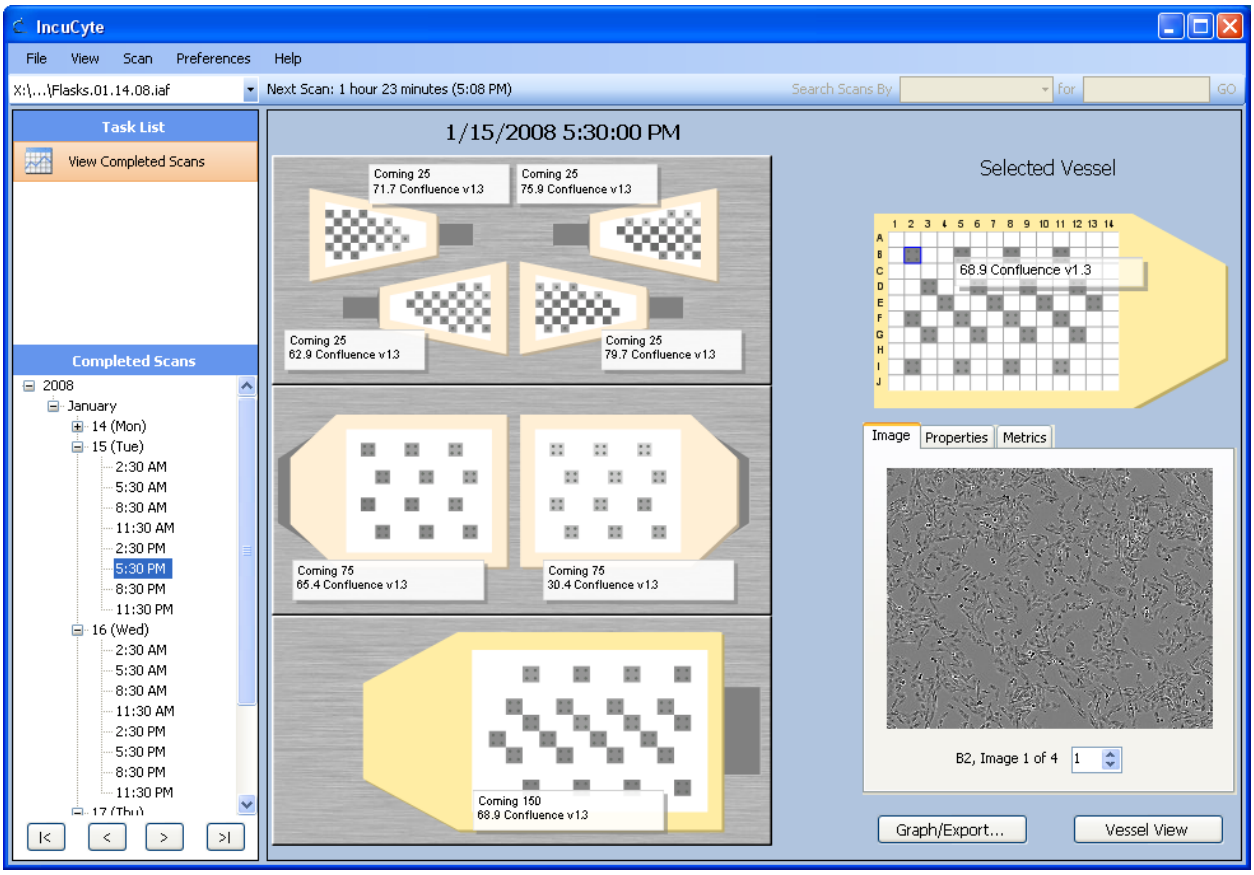


Figure 8-72. The Archived Scans Screen

Closing an Archive

To close the archive screen but not exit the IncuCyte™ program, select the Close Connection option from the file pull-down window at the top, left corner of the screen.

The Archive Folder and the Archive.iaf File

The data archive consists of two components: the .iaf file and the folder. The folder contains the actual data and the .iaf file contains the pathway to reach those data. Each is useless without the other. Whenever archived data are moved from one location to another, BOTH the folder and the .iaf file MUST be moved together. Additionally, if it is desired to rename an archive, be sure to rename the .iaf file and NOT the folder. If the folder is renamed, the data will no longer be accessible.

IMPORTANT: NEVER rename an archive folder. The .iaf file can be renamed, but NOT the folder. If the archive folder is renamed, then the archived data will no longer be accessible!

8.6.8 Logs Tab

All IncuCyte™ activities are monitored and stored, and they can be accessed using the Logs tab of the Administer IncuCyte™ screen (Figure 8-73). These logs can be consulted to investigate a variety of IncuCyte™-related issues. They can also be used by Essen BioScience towards the diagnosis of problems. Each of the logs can be exported by first selecting a log and then clicking on the Export button at the bottom left of the screen. Only the log that is currently selected will be exported. Therefore, multiple logs must be exported individually. Exporting logs may be required in the event of a problem, as Essen BioScience may request a copy of the log(s) via email to facilitate analysis.

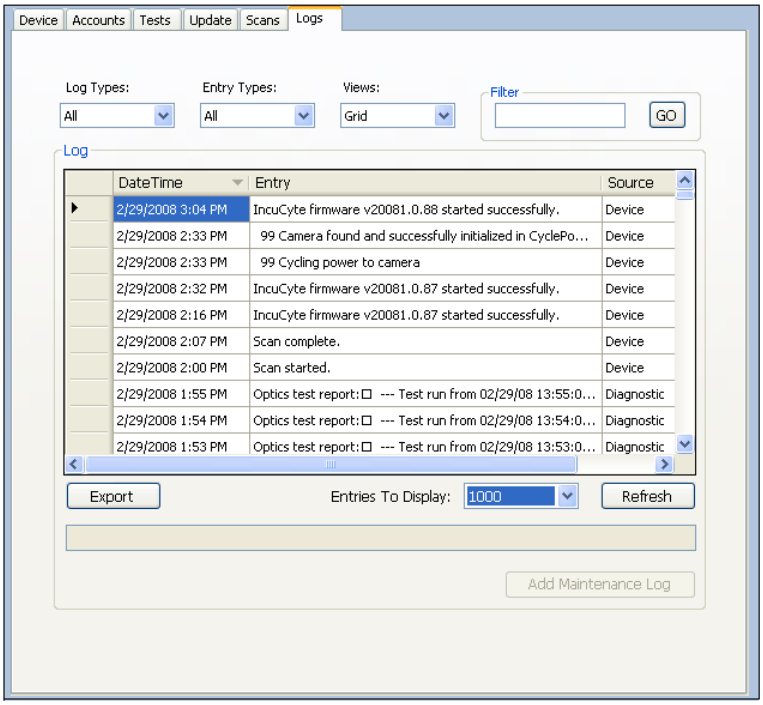


Figure 8-73. The Logs Tab

Any IncuCyte™ activities will be automatically updated into the Logs Section as they occur. However, if the Logs screen is opened up directly after an activity has occurred, or if the activity occurs while the Logs screen is already open, the change will not appear among the Log entries. To ensure that the most up-to-date information is being displayed, click on the Refresh button at the bottom right portion of the screen.

The Logs tab consists of three components:

- Log Types
- Entry Types
- Views

The Filter box is a search function that filters out all Log entries that do not match the criteria entered into the Filter box.

Log Types

Five types of logs can be viewed using the Log Types pull-down menu.

All

The All log contains the combined log entries from device, client, diagnostic and maintenance.

Device

The Device log records all device activities irrespective of the User. The start and stop times/dates of each scanning session are indicated as Scan Started and Scan Complete. Self-Test start and end times are also included. Some additional, physical manipulations of the instrument will also be recorded, including drawer opening and closing, and the pushing of the manual Stop button at the front panel. Although it may initially seem trivial, drawer opening and closing can be highly relevant under certain circumstances. Each time the drawer is opened and closed, it can cause a slight displacement of the images to be scanned. For example, if large image displacements are observed within a movie, consult the Device log to confirm that the drawer was not opened between scans.

Client

This log records all IncuCyte™ manipulations by Users. The Client log indicates the User name, time, date and a brief description of each action. Use the Client log as a resource for tracking all User-related IncuCyte™ activities.

Diagnostic

This log records the details and results of all diagnostic tests performed on the instrument. These include the Calibration and/or Confirm Tests, Optics Tests, and others.

Maintenance

When type Maintenance is selected, the Add Maintenance Log Entry button at the bottom of the screen will become active. Maintenance Log entries should ONLY be made by Essen BioScience employees during service calls. Users should NOT make any entries into the Maintenance log.

IMPORTANT: Users should NOT make any entries into the Maintenance Log Section.

Entry Types

For each Log Type, three log categories are available through the pull-down menu.

All

Choosing All displays all history affiliated with the selected Log Type, whether or not errors were associated with those events.

History

All IncuCyte™ activities that did not involve errors will be displayed for the selected Log Type.

Errors

Any IncuCyte™-related errors associated with the selected Log Type will be displayed under this category (Figure 8-74).

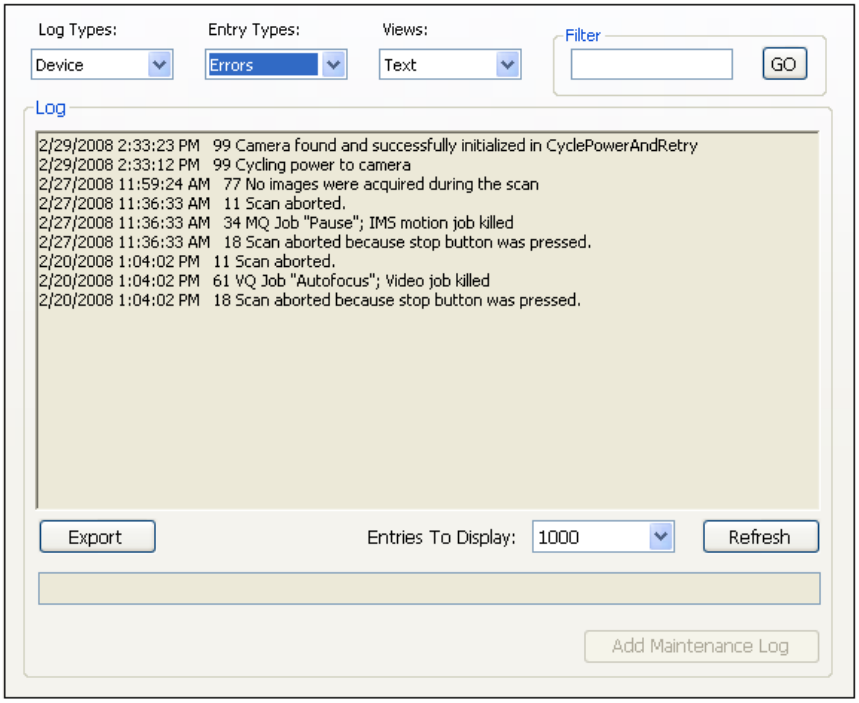

Log Types:
Device
Entry Types:
Errors
Views:
Text
Filter
GO
Log
2/29/2008 2:33:23 PM 99 Camera found and successfully initialized in CyclePowerAndRetry
2/29/2008 2:33:12 PM 99 Cycling power to camera
2/27/2008 11:59:24 AM 77 No images were acquired during the scan
2/27/2008 11:36:33 AM 11 Scan aborted.
2/27/2008 11:36:33 AM 34 MQ Job "Pause"; IMS motion job killed
2/27/2008 11:36:33 AM 18 Scan aborted because stop button was pressed.
2/20/2008 1:04:02 PM 11 Scan aborted.
2/20/2008 1:04:02 PM 61 VQ Job "Autofocus"; Video job killed
2/20/2008 1:04:02 PM 18 Scan aborted because stop button was pressed.
Export
Entries To Display:
1000
Refresh
Add Maintenance Log

Figure 8-74. Device Errors

The Errors log will be used primarily by Essen BioScience in the event of a problem. If an error appears to have occurred, such as a scheduled scan does not take place, consult the Device Errors log to see if an error has been recorded. Errors will be recorded with an error description, a numerical code, and the corresponding time and date. Examples of errors are displayed in Figure 8-74. In the event of a Device Error, the best approach is to restart the device and try again. If the error is persistent and cannot be eliminated, consult Essen BioScience for advice. In the event of a Calibration Error, check that the Calibration Tray is in the correct location and properly seated, and then retry the calibration procedure. If the error persists, consult Essen BioScience for assistance.

Views

The log contents can be viewed as a Text or Grid format. When the log is viewed in the Grid format, The Source of the entry is also displayed (e.g. Diagnostic, Device, Client) (Figure 8-75).

Use the Filter box to eliminate log entries that do not contain your word of interest (Figure 8-76). Note that only the information within the Entry Section can be searched - the logs CANNOT be filtered by Date, by Source, or by the IsError column. Log entries can be filtered in either the Grid or Text format.

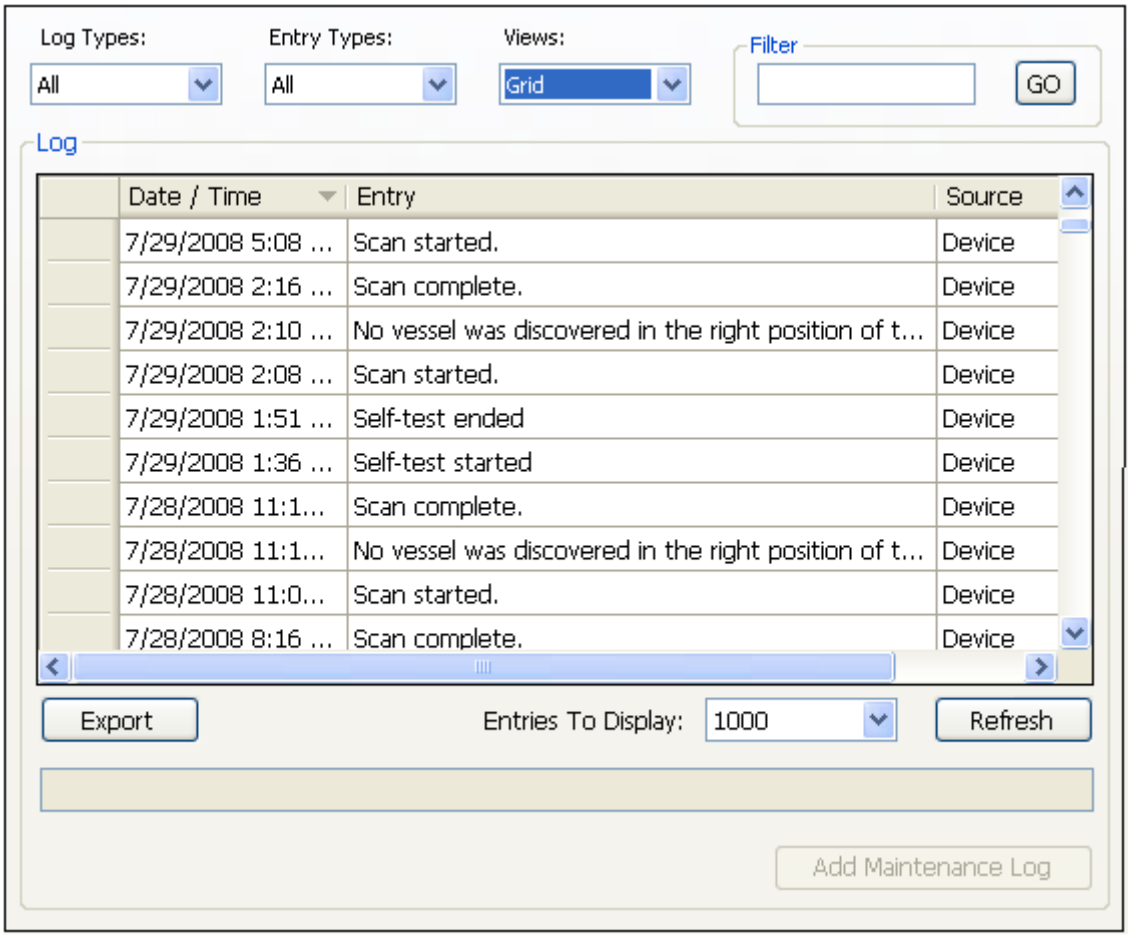


Figure 8-75. Log in Grid View. Use the blue scroll bar at the bottom of the screen to reveal the additional (hidden) Is Error column.

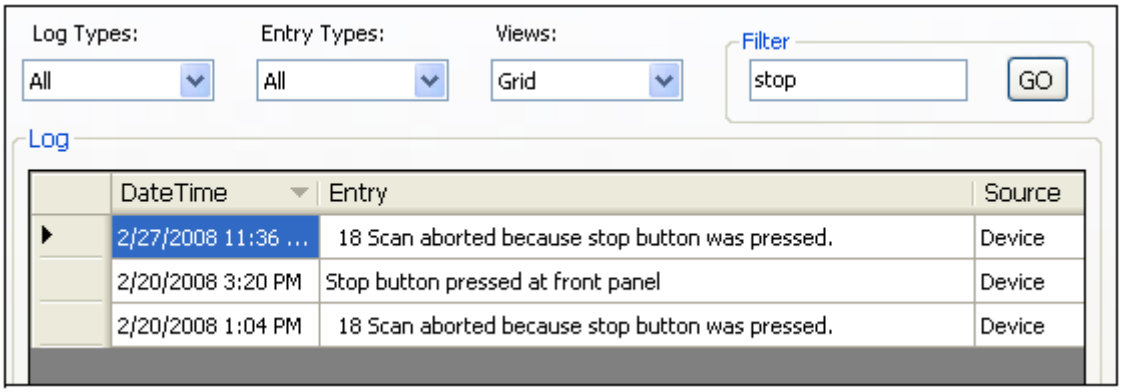


Figure 8-76. The Log entries were filtered using the word STOP.

8.7 View Completed Scans

8.7.1 Overview

When this screen is open, there is a Completed Scans box located beneath the Task List bar. The Completed Scans box lists the dates and times of all completed scans. Dates are preceded by a ⊞ or ⊟ icon. Selecting the ⊞ icon will open the next set of subdates/times available for that date. Selecting the ⊟ icon will close them again. To view the scan for a specific time, highlight the time itself. The corresponding scan will be displayed, and the date and time will also be indicated in bold at the top of the page. Scans can only be displayed one at a time.

At the bottom of the completed scans window (Figure 8-77, Figure 8-78, Figure 8-79), the arrow buttons can be used to browse through the scan times.

Figure 8-77. Scroll up and down one data/time at a time.

Figure 8-78. Jump to Last Scan.

Figure 8-79. Jump to First Scan.

In the center of the screen, the selected scanned vessels can be viewed. The three filled trays are indicated by black arrows in Figure 8-80 .

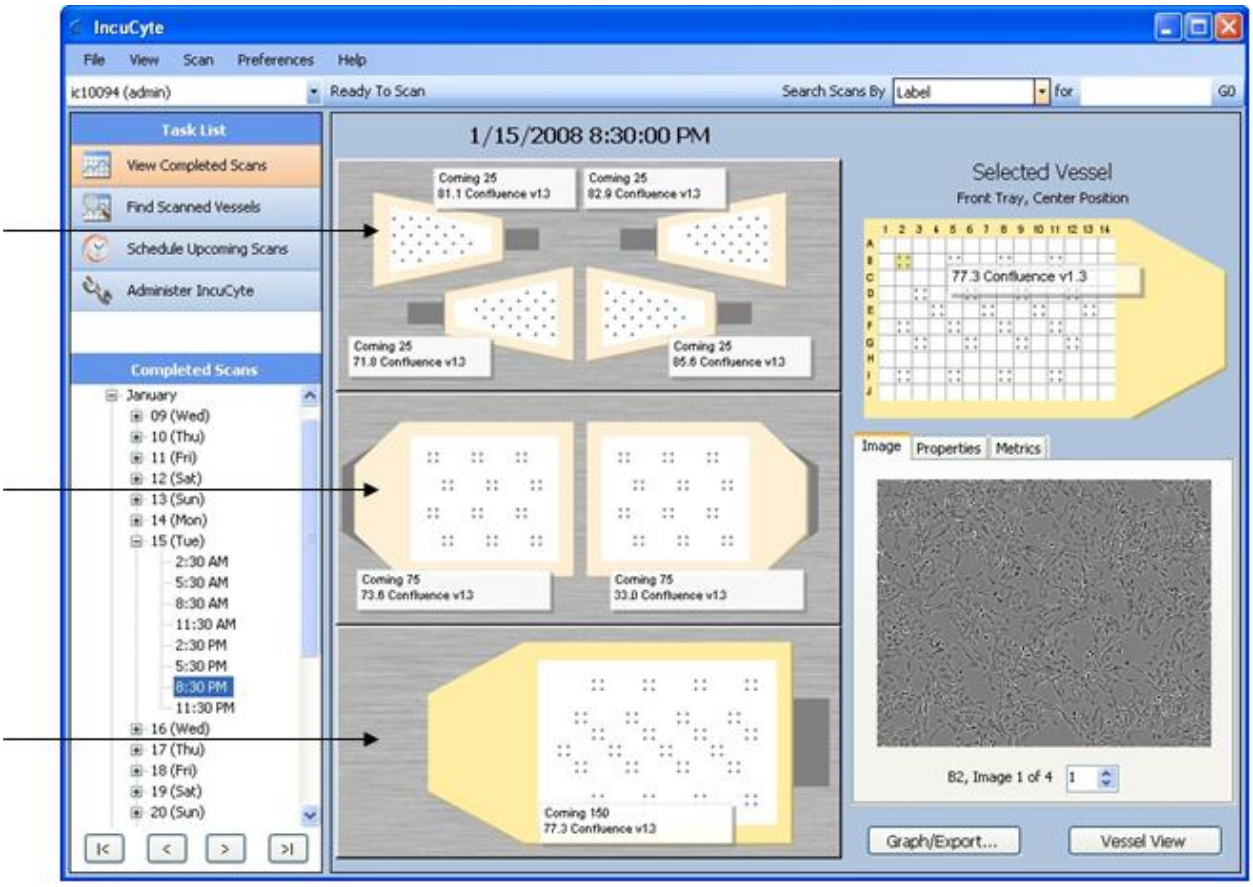


Figure 8-80. Trays are indicated by black arrows. Sector Shading is unselected, and the sectors are all white.

The following images/information will be visible on this screen:

- Each scanned vessel.
- Within each flask, dish, or well, the scan pattern is indicated as a pattern of dots.
- A box is displayed on each scanned vessel listing the vessel's properties. These properties can include the Label, Cell Type, Passage, Metric, and Sector Shading. The number of characteristics displayed within this box can be adjusted under the View pull-down menu at the top of the screen (Figure 8-81).

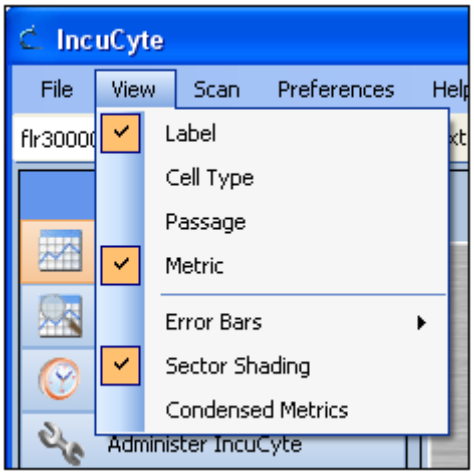


Figure 8-81. Select the characteristics displayed under the View Completed Scans Screen

- Sector Shading: A graphical representation of the sector or well Metric (this will generally be confluence) value can be viewed by selecting Sector Shading from the View pull-down menu (see Figure 8-81). Each sector or well will be shaded grey. The darker the shade of grey, the higher the Metric value. The View Completed Scans screen with active Sector Shading is displayed in Figure 8-82.

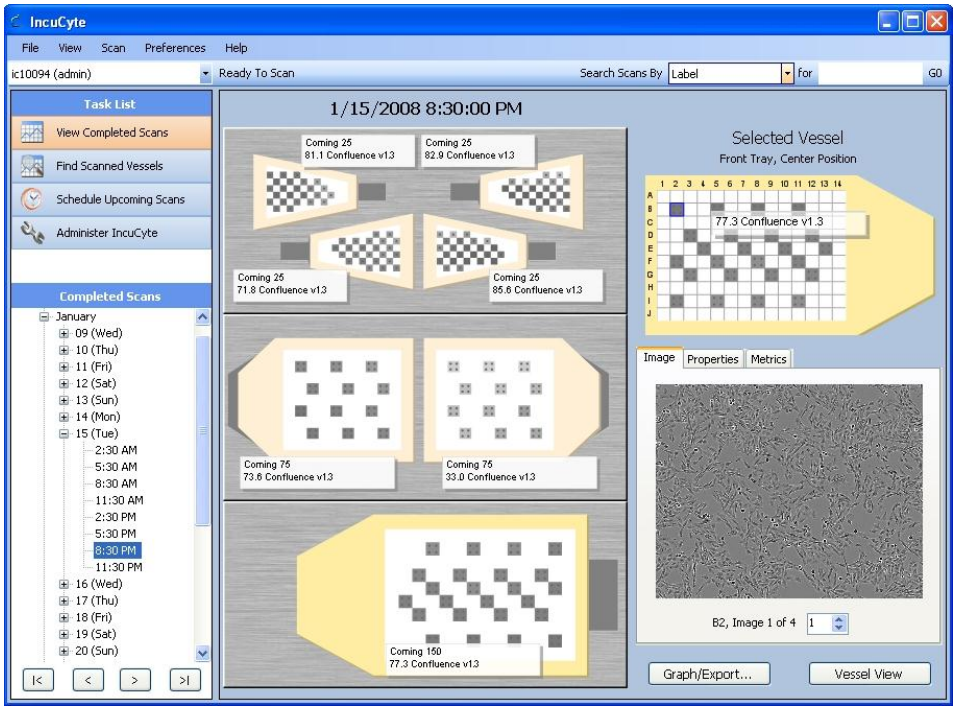


Figure 8-82. View Completed Scans Screen with Sector Shading selected. Because the selected Metric is confluence, darker shading represents higher confluence.

- Error Bars are discussed in Section 8.10.5 (page 187).
- Condensed Metrics are discussed in Section 8.7.2 (page 131).

If not all available spaces in the tray were occupied, then the spaces will contain no images of a vessel, and they will read Empty or Deferred (Figure 8-83).

- Empty: No vessel is set to scan in this position.
- Deferred: A vessel is in this position, but IncuCyte™ did not scan it at this time point.

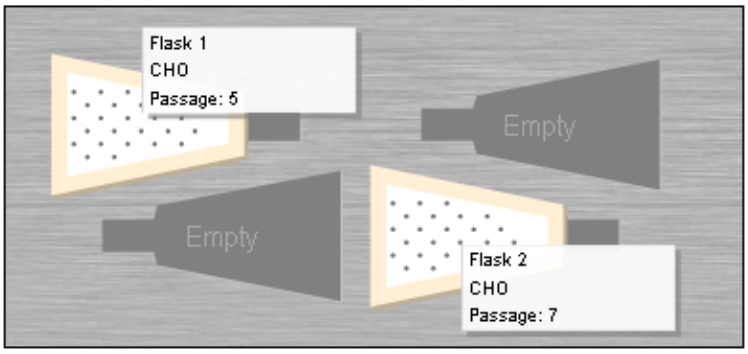


Figure 8-83. Empty flask spaces in an IncuCyte™ tray. Note the scan pattern indicated by dots within the flask.

8.7.2 Selected Vessel

Next to the vessels, is the Selected Vessel box. Highlighting a particular vessel on the tray will result in a larger, expanded version in this window. If no vessel is selected, the Selected Vessel box will be empty. See Figure 8-82. There are three tabs at the bottom of the Selected Vessel Box:

- Image
- Metrics
- Properties

Image Tab

Large views of some selected vessels are included in Figure 8-84 and in Figure 8-85. In the large view, the flask is divided up by squares into sectors. Sectors with dots in them represent sectors that were scanned. By highlighting sectors containing dots, you can view the image(s) of the cells in that sector when the Image tab is selected (as in Figure 8-84). In some cases, multiple images have been obtained for each sector or well. In that case, each image within the sector or well can be viewed by using the Image 1 of __ buttons below the image. In Figure 8-84 only one image was obtained per sector (Image 1 of 1), so the scroll box is inactive.

The image can be viewed in a full-screen version by double-clicking on it with the mouse.

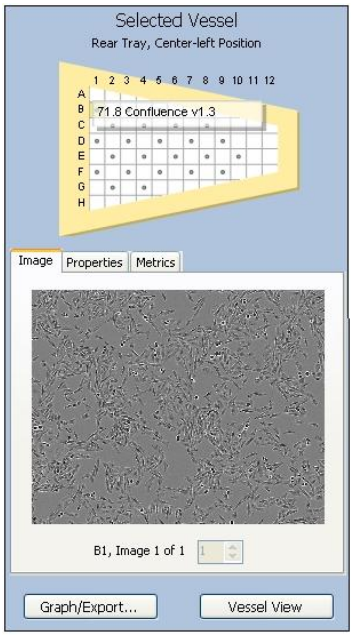


Figure 8-84. Flask area is divided into Sectors.

The selected well or sector will also display a window indicating the corresponding Metric (in this case confluence).

Note: Sectors are only used in flasks and dishes, NOT in microplates.

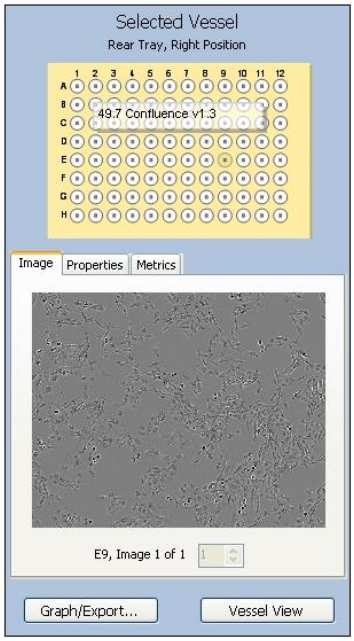


Figure 8-85. Microplate area is divided into Wells.

Properties Tab

The Properties tab displays the properties associated with the selected vessel. Properties include:

Scan Type: This will usually be standard. See Section 8.5.5, page 73.

Label

Cell Type

Passage Number

Vessel Type

User Name: This refers to the user who set the scan

Add Notes: Add any relevant Notes

Notes: This is an inactive window that displays Notes that have been added in the. Note that have been previously added cannot be edited.

Some vessel Properties can be edited. These include the vessel Label, Cell Types and Passage number. New Notes can be added, but previously added Notes cannot be edited. To edit properties, enter the new information and press the Update button at the bottom of the window.

EX

Properties Tab EX

The vessel Properties can be viewed but not populated or updated through the IncuCyte™ EX software. These boxes are remotely populated by the automated cell handler. The vessel Type, Label, etc. displayed in Figure 8-86 were filled in via the remote interface. See Section 8.5.6, page 75.

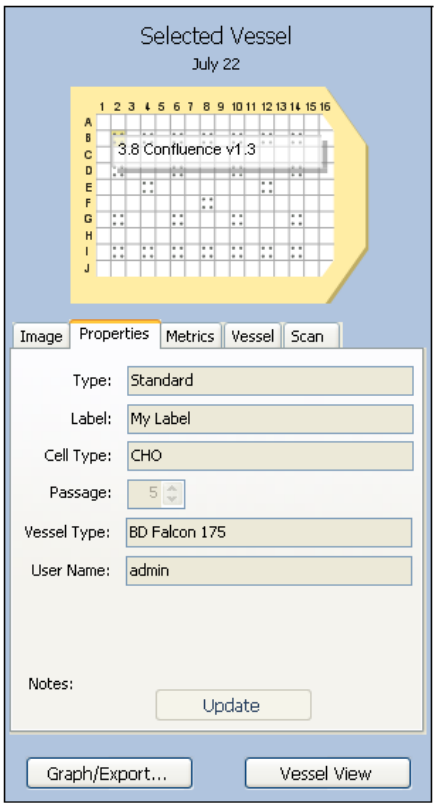


Figure 8-86. Properties EX

Metrics Tab

The Metric that will be primarily used is Confluence. The remaining Metrics: Image Mean, Focus Position, and Exposure time are diagnostic tools used to confirm that the software is functioning within specified parameters. These tools will not be relevant to the User under normal circumstances. Whichever Metric is selected here will be displayed on the View Completed Scans screen.

NOTE: Metrics other than Confluence are ONLY available at the Administrator permission level.

Definitions of the Metrics Spreadsheet

- Sector or Well: Denotes the sector or well to which the data in that column belong.
- Mean: Displays the Mean values for all images in each well or sector.
- Median: Displays the Median values for all images in each well or sector.

NOTE: If there is only one image per Well or Sector, then the Mean and Median values will be the same

- Std.Dev: Denotes the global Standard Deviation of all sectors/wells for that vessel.
- 1 of __: Indicates the Metric value for each image of each sector or well.
- Min and Max: Displays the minimum and maximum values for that vessel.

Condensed Metrics

The Metrics can be viewed both in a full or a condensed format. In the condensed format, the individual image data are not displayed. Toggle between the condensed and non-condensed view format by selecting it from the View pull-down menu at the top of the screen. See Figure 8-81, page 126.

Tasks Available by Right-Clicking on a Selected Vessel

Right-click on a vessel in the View Completed Scans screen, and a selection of Tasks related to that vessel will become available. These Tasks include:

View Vessel: Select this Task to open the Vessel View window

Graph/Export: Graph/Export data from the vessel. See Section 8.10, page 165 and Section 8.10.4, page 172.

Archive Vessel: Archive the vessel. See Archive Vessels, page 114.

Delete Vessel: Delete the vessel. See Delete Vessels, page 116.

8.7.3 Vessel View Overview

The Vessel View offers expanded tools related to viewing and manipulation of images/data. There are 6 ways to open the Vessel View window.

- Press the View Vessel button under the Image/Metrics/Properties box (Figure 8-82).
- Double-click on a vessel in the Find Scanned Vessels Screen.
- Double-click on a vessel in the View Completed Scans Screen.
- Select a vessel in the View Completed Scans Screen and then choose Vessel View from the Scans pull-down menu at the top of the screen.
- Right-click on a vessel in the View Completed Scans screen.
- Double Left-click on a point from an IncuCyte™ graph.

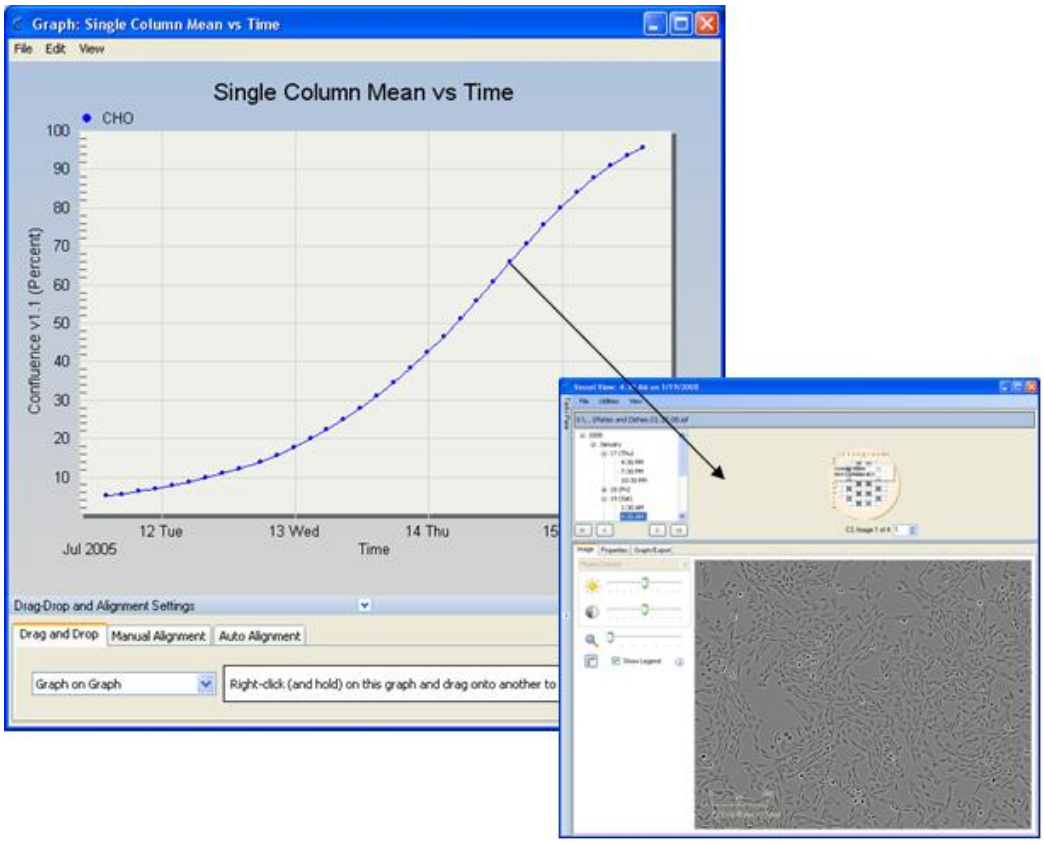


Figure 8-87. Double left-click on one of the points on the IncuCyte™ graph to open the Vessel View window for that point.

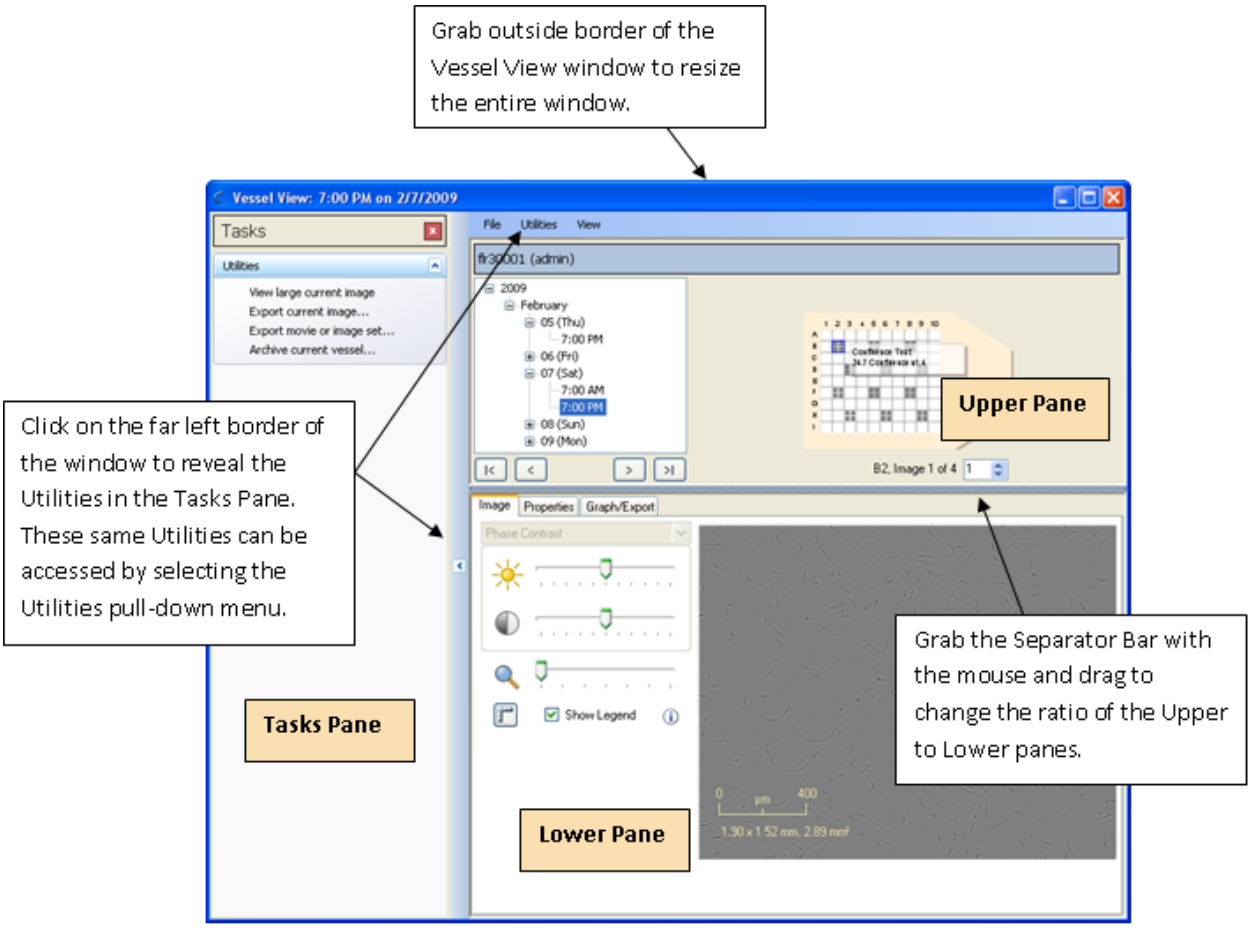

Grab outside border of the Vessel View window to resize the entire window.
Vessel View: 7:00 PM on 2/7/2009
Tasks
Utilities
View large current image
Export current image...
Export movie or image set...
Archive current vessel...
File
Utilities
View
Upper Pane
Image
Properties
Graph/Export
Show Legend
Click on the far left border of the window to reveal the Utilities in the Tasks Pane. These same Utilities can be accessed by selecting the Utilities pull-down menu.
Tasks Pane
Lower Pane
Grab the Separator Bar with the mouse and drag to change the ratio of the Upper to Lower panes.

Figure 8-88. The Vessel View Window

A variety of tasks can be selected using the Utilities pull-down menu or by clicking on the far left border of the Vessel View window to open the Tasks Pane. See Figure 8-88.

Tasks Pane

Right or left click on the far left border of the Vessel View window to open the Tasks Pane. The Utilities visible within the Tasks Pane can also be accessed using the Utilities pull-down window at the top of the Vessel View window. Most of these utilities will be described elsewhere.

- View Large Current Image: Increase the current image to full screen size.
- Export Current Image: See Section 8.7.4, page 136.
- Export Movie or Image Set: See Section 8.7.4. page 136.
- Archive Current Vessel: See Section 8.6.7, Archive Vessels, page 114.

Time Tree

To the left of the selected vessel, there will be a box displaying scan times. The blue bar above the box displays the instrument number (IC10094 in Figure 8-89) and the User name (MeMe in Figure 8-89), or the folder to which data have been archived (to be discussed later). By selecting a time, the corresponding image will be displayed. The Vessel View Time Tree displays ONLY the scans that occurred for the selected vessel.

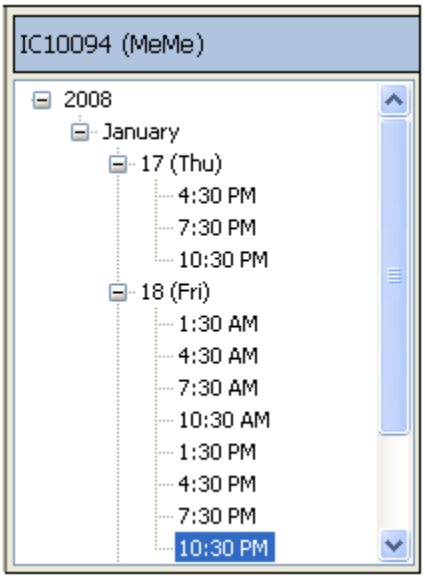


Figure 8-89. Time Tree. User: MeMe, Instrument: IC10094, Selected time: 10:30pm

Selected Vessel

The selected flask, dish or microplate is displayed to the right of the Time Tree. Use the Selected Vessel to view the images in different sectors/wells by selecting them with the mouse.

EX

Selected Vessel EX

In the case of the IncuCyte™ EX, the barcode readout is displayed under the Selected Vessel instead of its position in the drawer. In Figure 8-90 the barcode label is ACF7234523HZ. The barcode will also be displayed in the EX Vessel View window.

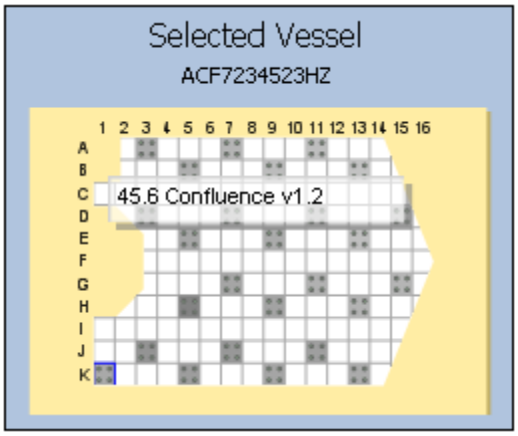


Figure 8-90. The Selected Vessel Barcode in EX

Image Tab

The image for the currently selected well or sector is displayed under the Image tab. Use the brightness and contrast sliders to adjust image quality.

Double click on the Image in the Vessel View window, use the Large Image Shortcut icon, or select the Utility: View Large Image to increase the currently displayed image to full screen size (Figure 8-88).

Zoom

Use the Zoom Slider bar to zoom into and out of the image. Dragging the slider bar will zoom to the center of the image; however, the area of the zoomed image being displayed can be changed by grabbing the image and dragging it with the mouse. Alternatively, it is possible to zoom directly to a specific area within the image by scrolling the mouse wheel over the area of interest.

Show/Hide Legend

Select the Show Legend box to display the size ruler at the bottom, left-hand corner of the image. Uncheck the box to remove the ruler. The legend can be viewed in the Vessel View window, but it will not be included in movies or exported images. In Figure 8-88 the Legend is turned on.

Measurement Mode

Use Measurement Mode to make linear measurements within the image. To turn Measurement Mode on or off, click on the ruler icon next to the Show Legend box at the bottom of the Vessel View window. See Figure 8-91 and Figure 8-88.

Figure 8-91. The Measurement Icon is a blue ruler located to the left of the Show Legend box.

When Measurement Mode is on, the background color of the ruler icon will change color, and a dark bar will appear in the bottom right-hand corner of the image. Use the mouse to draw a line across the image. The length of the line (in µm) will appear in the dark bar at the bottom of the screen. Measurement Mode can be used in full-sized and zoomed images.

Mouse-Over Pixel Location Readout

Holding the mouse over any region of the image will display the exact coordinates for the currently selected pixel.

Properties Tab

Selecting this tab displays the properties of the selected vessel. These are the same properties as discussed in Properties, page 129 with the addition of one additional Property: Vessel Location.

Properties Tab EX

EX

Recall that vessel Properties can be viewed but not added or edited in the IncuCyte™ EX . The built-in Properties (Cell Type, Passage, etc) are available under the Properties tab. See Section 8.5.6, page 75 for more information.

Graph/Export Tab

Displays Metrics. Display Metrics and access Graph/Export functions. See Section 8.10, Preparing to Graph, page 172 and Section 8.10.4. Export Metrics, page 172. These same functions can also be accessed using the Vessel View utilities pull-down menu. See Figure 8-95.

Vessel and Scan Tabs EX

EX

Two additional tabs: Vessel and Scan are available on the IncuCyte™ EX . See Section 8.5.6, page 75 for more information on these tabs.

8.7.4 Vessel View FLR

FLR

The Vessel View window for fluorescently scanned vessels contains expanded options and functionalities relative to vessels scanned non-fluorescently. See Section 9, IncuCyte™ FLR, page 201 for information regarding viewing and manipulation fluorescently scanned images.

8.7.5 The Plate Map Editor

To assist in experimental design and data analysis, the IncuCyte™ software includes a Plate Map Editor that allows the User to custom-design a plate map for any microplate-based experiment. The User may design and save a plate map at any point before or during an experiment, or after the experiment has been completed. Access to the Plate Map Editor is available from four locations:

- Main IncuCyte window: Select “Plate Map…” in the File menu at the top of the window

  - From this location the User may save a plate map to a file, but not to an individual vessel.
- Schedule Upcoming Scans | Properties tab
- Vessel View | Properties tab
- The User may open the Plate Map Editor independently of the main IncuCyte software by browsing for the IncuCyte GUI program group via the Windows "Start" menu in the lower left of a Windows desktop.

With the Plate Map Editor open, the default screen is set to a blank 96-well plate layout (Figure 8-92. **The Plate Map Editor**). To change the plate layout, click on the New Plate Map (blank sheet) icon on the menu bar at the top-left of the window. The Plate Map Editor supports plate layouts from 6-well to 384-well formats. To load a plate map already saved to a file, click on the Open (folder) icon on the menu bar and browse to the file.

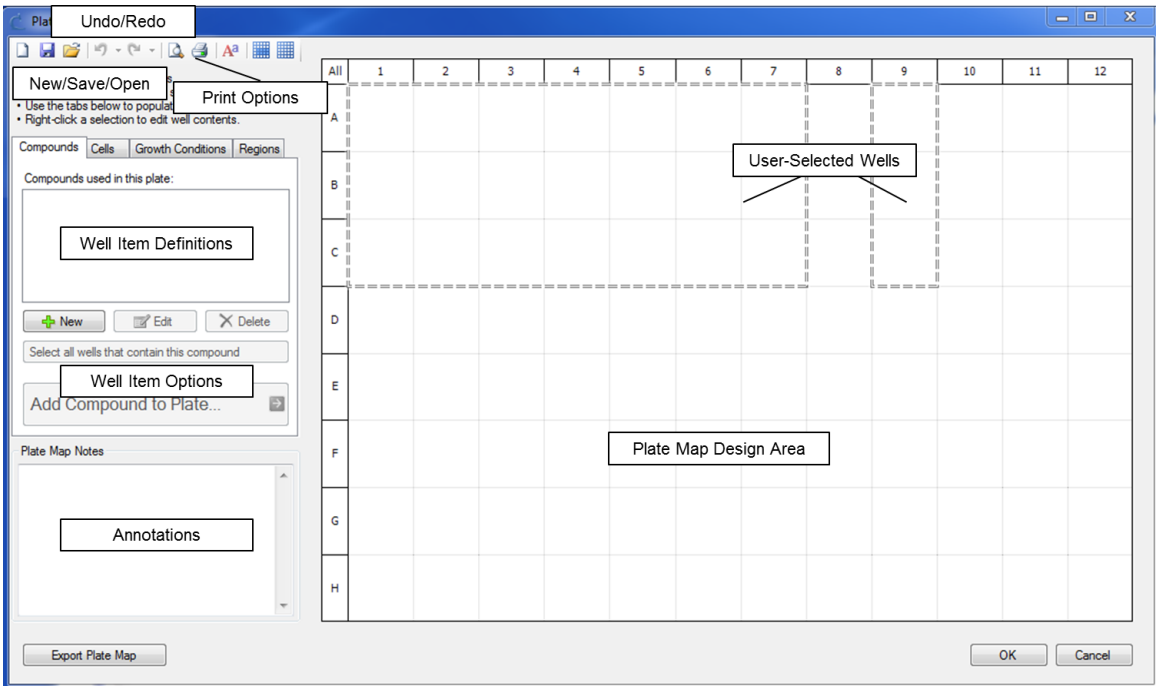


Figure 8-92. The Plate Map Editor

Selecting Wells

To add "well items" (cells, compounds, *etc.*) to a well or grouping of wells, the desired area must first be selected on the plate layout. Left-click on a well, or click-and-drag over a group of wells, to select a specific area of the plate in which to work. Wells may be deselected in the same manner. To easily select or deselect entire rows or columns, click the row/column label along the border of the plate. Select and deselect all wells in the plate by clicking on the "All" button on the top-left of the plate. Immediately to the left of the "All" button is a "blank plate" icon that will also deselect all wells (Figure 8-92).

Adding Well Items

Before any information can be applied to a well on the plate map, it must first be loaded into one of three lists to the left of the plate map. "Compounds" are any treatments to a well where concentrations or dilutions must be specified. "Cells" specifies type, passage, and seeding density for cells present on the plate. "Growth Conditions" are additions such as growth medium where concentrations of a reagent will not vary or need not be specified on the plate map.

Compounds

To add a compound to the plate map, click the "New" button below the (blank) compound list. In a new window, enter a name to appear on the plate map and, optionally, a longer description to appear in the list of compounds (Figure 8-93). Additionally, select a color in which the compound will appear on the plate map; when creating a dilution of a compound, higher concentrations will appear as darker shades of the indicated color.

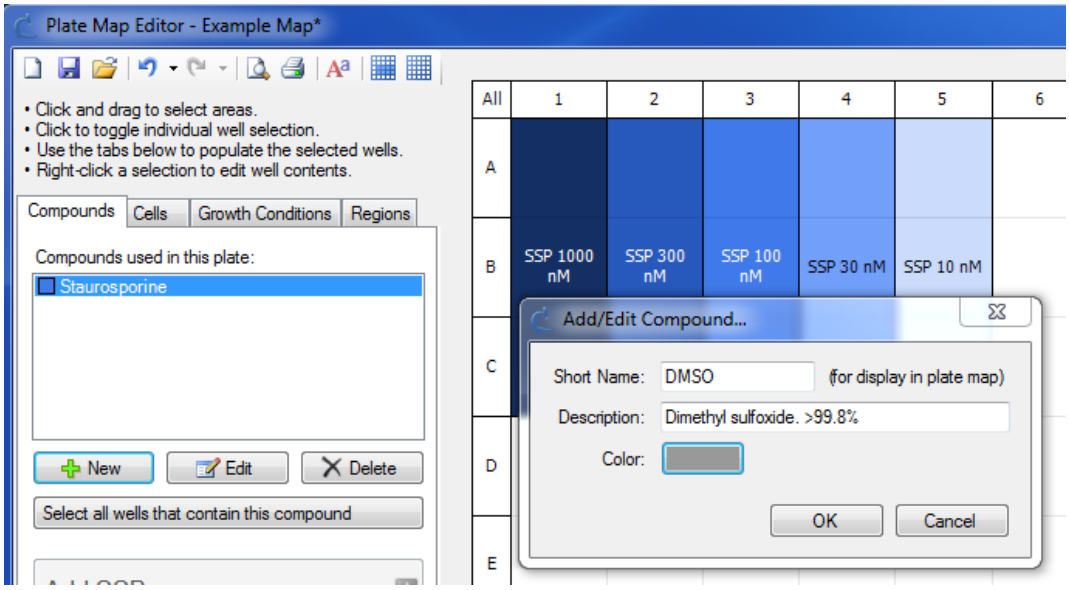


Figure 8-93. Adding Well Items to the Definitions lists.

After creating a compound description, select the desired area of the plate and click "Add ___...". A new window will appear allowing the User to select a desired concentration and, optionally, a dilution series. First, select the units in which concentration will appear by clicking the drop-down menu at the top of the window (the default setting is "mg/mL") and enter a numerical concentration to the left. If only a single concentration is desired, click "OK" and the compound will appear on the plate map.

To create a dilution series, check the box next to "Create a dilution" to activate dilution options. In the drop-down menu immediately to the right, select "divide by" or "subtract" and enter value by which to dilute. Alternatively, select "manually enter" to enter a range of concentrations separated by spaces or

semicolons (1000; 300; 100; 30…). To arrange the dilution series on the plate, select the direction in which the dilution series runs by selecting a direction under "Dilute wells…" and the direction in which the replicates run. If, for example, a dilution series runs from left-to-right and the User wishes the replicates to be arranged horizontally, a box will appear allowing the User to specify how many wells exist at each concentration (singlets, duplicates, triplicates, *etc.*). Once the dilution series is specified, click "OK" and the compound will appear on the plate map in the specified series.

Cells

Add a cell type in the same manner as a compound (Figure 8-93). Once a region of the plate is selected, click "Add ___..." to open up a new window. Here, specify passage number and seeding density, and click "OK" to add the cell type to the plate map.

Growth Conditions

Once a growth condition is specified in the list, simply click "Add ___..." to add it to the plate. Because the Growth Conditions category is used for conditions where no concentration or dilution series is specified, clicking "Add ___..." will add the condition directly to the plate with no intervening options.

Adding Multiple Well Items

To sequentially add multiple items to a grouping of wells (*i.e.* cells, growth conditions, compounds), select the plate map icon, second-to-the-right on the top menu, that reads "Keep Selection After Adding to Wells". Normally after adding an item to a well or group of wells, that grouping will automatically be deselected. With the "Keep Selection…" icon activated, the selected grouping will remain so after addition of a well item.

Deleting/Editing Well Items from Lists

Once the three lists are populated, the User may edit or delete items from the lists. Editing an item will open the same window as when adding an item to a list, only the fields in the window will already contain the information for the selected item. Edit the desired fields (Short Name, Description, Color) and hit "OK" to save the changes. To delete an item from a list, simply click on the item, hit "Delete," and "OK". Note that this action does *not* delete any instances of the item already added to the plate map.

Editing Well Contents

Once a well or group of wells are populated, the User can edit its contents and appearance. To edit well contents, first select the desired wells by left-clicking over them, then right-click within the selected region to bring up the editing window. From here, the User can create a custom description of the selected

well(s), delete items, and change the color scheme of the well(s). Alternatively, use the "Undo" and "Redo" buttons in the menu bar at the top-left of the window if a mistake is made while editing a well's contents. In addition, text size/font/style can be changed using the font button in the menu bar.

Creating and Using Regions

Regions on a plate map allow the User to define a specific set of wells (*e.g.* a dilution series), which aids in graphing and exporting data from an experiment. Once defined, Regions appear in the Graph/Export window (See Graph/Export Tab**,** page 136) in the "Regions" drop-down list and allow graphing/exporting of a custom grouping of wells. Any replicates defined within the region are automatically detected and can be grouped by selecting "Replicates" in the Graph/Export "Group" drop-down list. For example, the User can create a region such that an entire dilution series, along with negative and positive controls, appears on the same graph without the need to drag-and-drop individual plots onto one graph. In addition, the labels on individual wells or groups of wells ("replicates") will automatically appear in the figure legend of the graph.

To create a Region on a plate map, simply select the desired area of the plate in the Plate Map Editor and click on "Save Selection as Region…" under the Regions tab on the left side of the window (Figure 8-94. **Creating a custom Region.**). Enter a name for the region and hit "OK" to save the region.

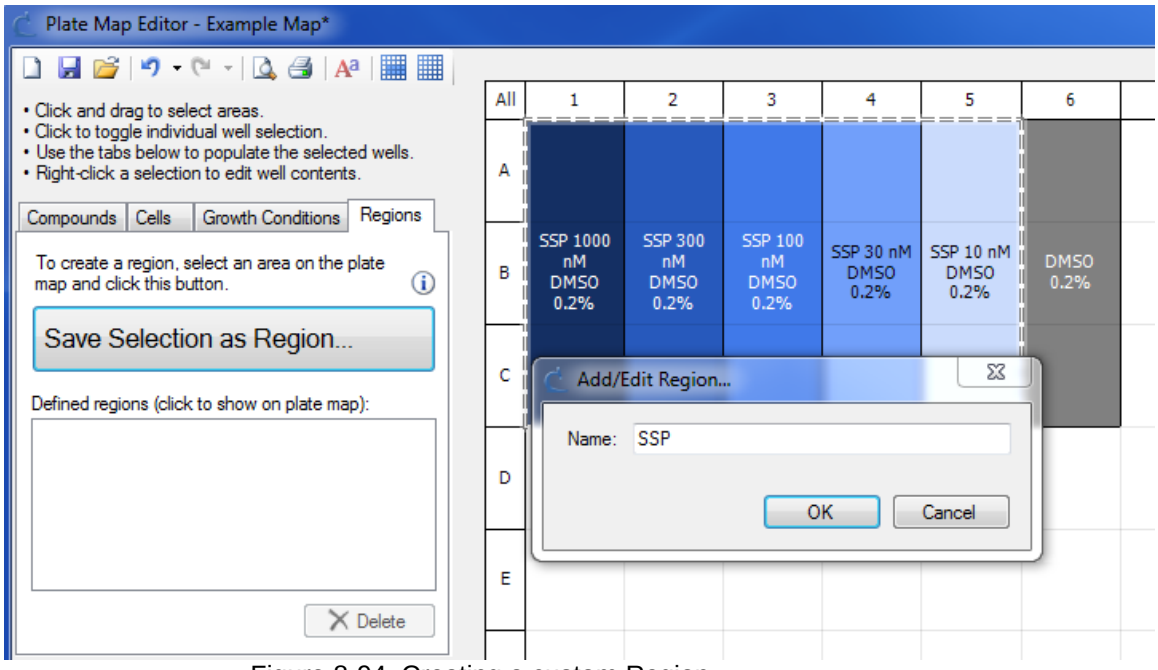


Figure 8-94. Creating a custom Region.

To create a graph of or export data from a saved Region, go to the Graph/Export window (from the View Completed Scans screen or the Vessel View window) and

find the Region in the “Regions” drop-down list (Regions created from the Plate Map Editor will be highlighted in blue). Select “Replicates” from the “Group” drop-down list to view each group of replicates (*e.g.* a specific drug concentration) as its own plot on the graph or to arrange exported data into replicates.

Printing a Plate Map

At any time during the creation of a plate map, the User may print a hard copy of the map – to put in a lab notebook or to use while in the lab, for example. The “Print” and “Print Preview” buttons are both found in the menu bar at the top-left of the Plate Map Editor. In addition to the plate map, the hard copy will also include any notes written in the “Plate Map Notes” section below the Well Item lists in the Plate Map Editor.

Exporting an image of the Plate Map

In addition to simple print functions. Users can also export Plate Map images using the Export Plate Map button at the bottom left hand corner of the editor. This brings up a selection menu where the user can select from 4 different file formats (PNG, TIFF, JPEG, BMP) and 5 different predetermined sizes.

8.7.6 Export Images and Movies

Images can be exported either individually or in bulk. Movies can be generated using a sequence of images within a selected time frame. Both Image and Movie export functions need to be accessed through the Vessel Window by:

- Selecting the Utilities Pull-Down menu, or
- Revealing the individual Utilities in the Vessel View Tasks Pane (see Figure 8-95)

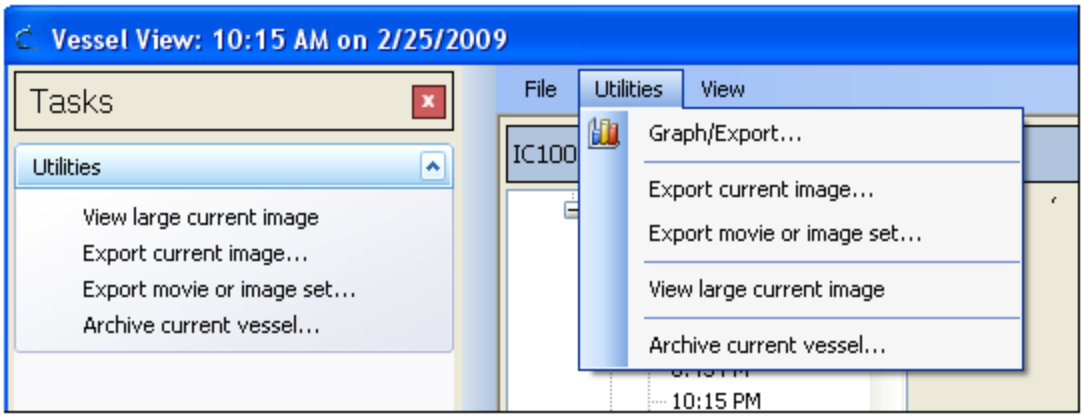


Figure 8-95. A cutout from the Vessel View showing the utilities accessed by the Utilities pull-down menu or in the Tasks Pane (left).

Export Images Overview

Images can be exported as either a JPEG or TIFF file. The Image can be exported as one of three image types:

- Phase Contrast using the current display settings (JPEG)
- Phase Contrast Original (JPEG)
- Phase Contrast Original (8-bit TIFF)

Phase Contrast using the current display settings (JPEG)

This function will export the image using the currently displayed image settings; for instance, if the brightness and/or contrast settings have been adjusted. Zoomed images can also be exported by selecting the Maintain CurrentView/Zoom check box, but note the special circumstances that might apply. See Figure 8-96. Export of images using Current Display Settings is only available in the JPEG format.

Phase Contrast Original (JPEG)

Export the image as it was originally acquired by the IncuCyte™ microscope in the format of a JPEG file.

Phase Contrast Original (8-bit TIFF)

Export the image as it was originally acquired by the IncuCyte™ microscope in the format of a TIFF file. Note that TIFF images can only be exported as originally acquired.

NOTE: TIFF images can only be exported as originally acquired.

Images in addition to the image currently displayed in the Vessel View window can also be exported.

Export Current Image (Single Image)

To export only the SINGLE image that is currently displayed in the Vessel View window, select Export Current Image… A new window will open. See Figure 8-96. Select the appropriate image type in the Export Single Image window using the Image Type drop-down menu.

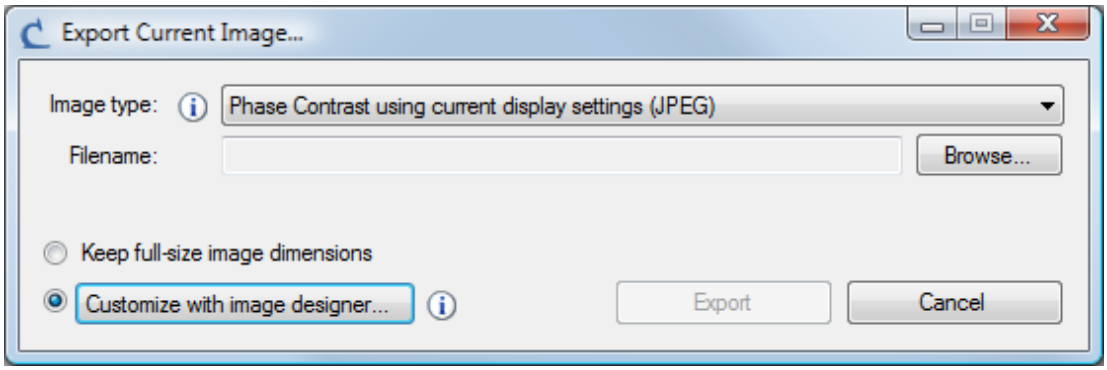


Figure 8-96. Export Current Image Window

Export utilizing the "Phase Contrast using Current Display Settings" will export an image that maintains the brightness and contrast settings currently displayed in the Vessel View. By selecting the "Keep full-size image dimensions" option, the entire image acquired at its native size is exported using the current brightness and contrast settings. Further customization of the export image is available by using the "Customize with image designer…" tool. This button launches a new window that allows the user to customize the cropping and zoom level as well as to add a legend and timestamp if desired. With the legend option, a scale bar and field of view dimensions will appear in the exported image as they do in the Vessel View.

Figure 8-97 shows a Vessel View including both the "Export Current Image" and "Export Designer" windows. Open "Export Designer" window by clicking the "Customize with image designer…" button in the "Export Current Image" window. Here, the full size image is cropped as it appears in the Vessel View window, whether viewed full-size or zoomed-in. The cropping boundaries can also be changed in the current window by clicking on and dragging the red boundary lines within the preview window. Similarly, the cropped rectangle can be moved by clicking and dragging within the current boundaries. The final size of the cropped image in pixels is displayed below the "Scaling" control. Use this control to adjust the size and resolution of the final image. As the scaling increases, the final size increases, and vice versa. This allows you to customize the final image size, which can be very convenient when exporting data for presentation formats. By increasing the scaling, you can digitally zoom in on a small cropped area.

NOTE: The sizes of both the scale bar and timestamp (in pixels) are fixed and will thus appear differently depending on scaling. Experiment with the "Scaling" option to see how this affects both the timestamp and legend on the final image.

Perhaps the most important tool found in the Export Designer is launched via the "Output Preview" button in the lower left corner of the Export Designer. We *strongly* encourage you to preview your customization settings prior to export!

The preview allows you to view the image at the exported size as well as in a full screen mode that fills your monitor. Once you are satisfied with the customization, click “OK” to exit the Export Designer. To export with the customization settings established in the Export Designer, make sure that the radio button next to the “Customize with image designer…” button is selected prior to clicking the “Export” button. One final note: Figure 8-97 shows a selected image type of “Phase Contrast using current display settings”, but other image types (like “Phase Contrast Original”, for example) can also be customized prior to export.

After the Image Type has been selected, browse to the file destination and select the Export button.

Remember that the current image can also be dragged and dropped from the Vessel View window by grabbing it and dragging with the mouse. Dragged and dropped images will always be saved in the format of a JPEG file.

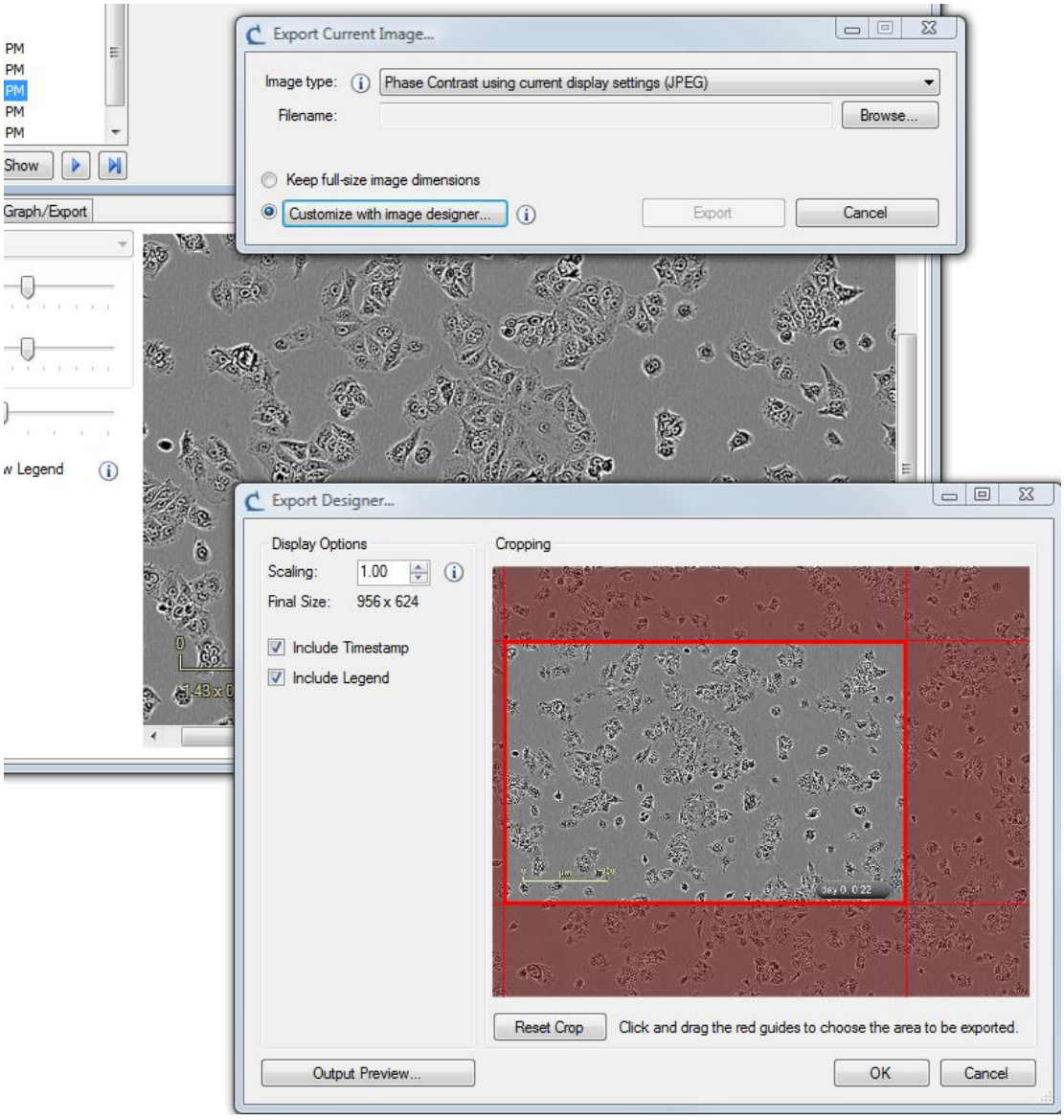


Figure 8-97. The Export Current Image window with Export Designer open.

Export Image Set (Multiple Images)

It is possible to export all or a subset of all the images obtained for a particular vessel in bulk format by selecting the Utility: Export Movie or Image Set. See Figure 8-98. This window displays the selected Vessel and its corresponding Time Tree. The window is divided into two main Sections: Export Time/Area Range and Save Location and Options.

Note that one or more possible images from each well or sector can be selected for export. The image number in the check box refers to which image will be exported. It does NOT refer to the total number of images that will be exported. Thus, if only the checkbox for image 3 is selected, then only image 3 will be exported for each selected well/sector and/or time point. That is, only ONE

image will be exported per sector or well, and that image will always be image number 3. To export all three images for each well/sector, the checkboxes for all three image numbers must be selected.

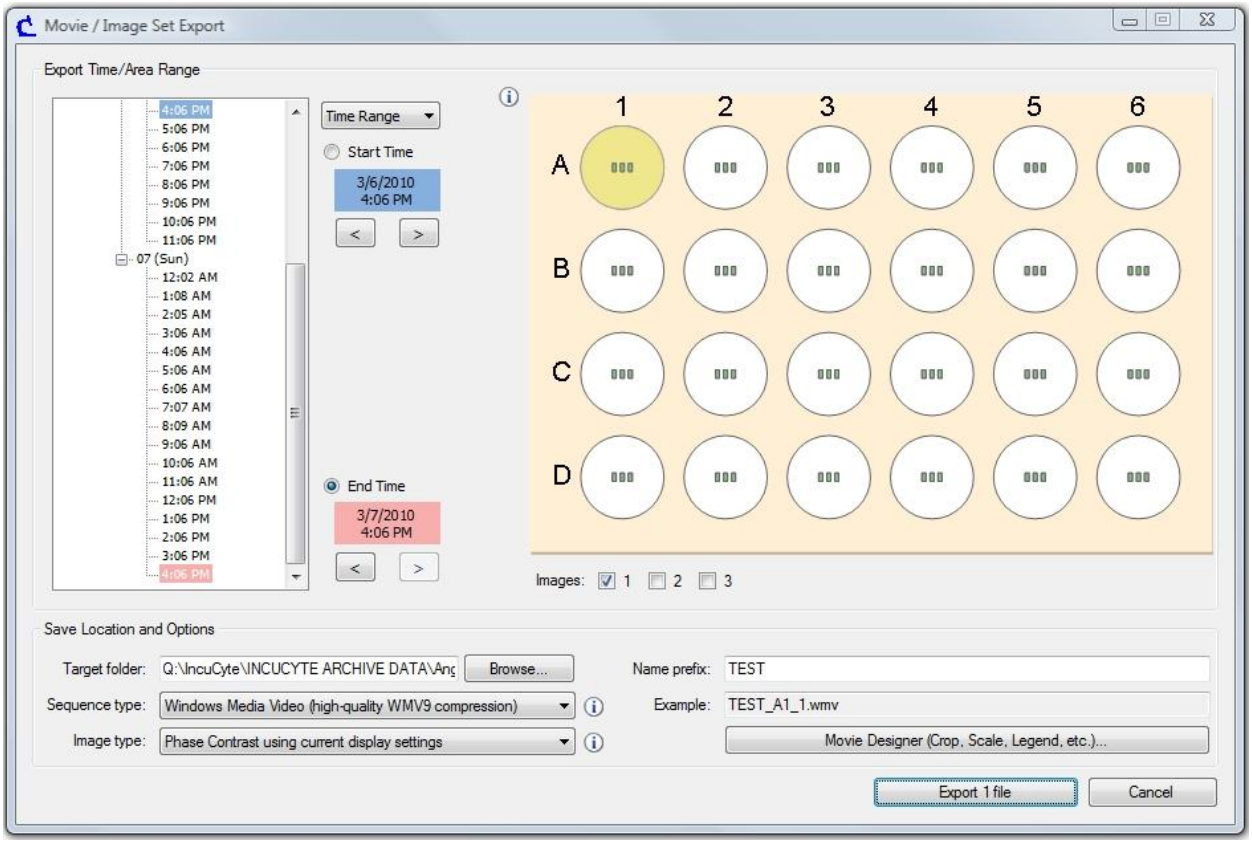


Figure 8-98. Movie/Image Set Export Window

Select Wells/Sectors for Image Export

One or more wells/sectors can be selected for image export.

Select one or more wells/sectors by clicking on them individually with the mouse.

Select a region of the vessel by clicking and dragging with the mouse.

Deselect one or more individual wells/sectors by holding the “Alt” key and then clicking with the mouse.

Deselect a region of the vessel by holding the “Alt” key and then clicking and dragging with the mouse.

Right-click on a well/sector to activate additional options for selection/deselection. See Figure 8-99.

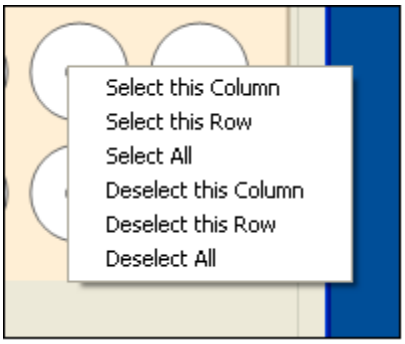


Figure 8-99. Right-click for More Options

It is possible to individually select up to 9 images per well/sector for export if the scan pattern includes 9 images or less. However, it is also possible to set a scan pattern that includes more than 9 images per well/sector. In this case, there will be two options for image export:

Export all Images

Export Image __ of Total

Single Time Image Export:

Select Single Time instead of Time Range. See Figure 8-100. Then, use the arrow buttons to scroll to the appropriate time, or use the mouse to select a time.

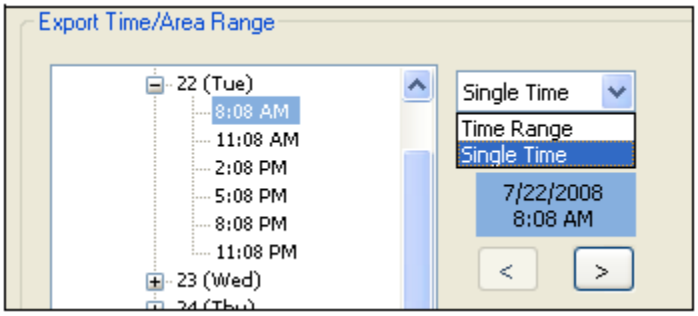


Figure 8-100. Select Single Time for Image Export

Select the sectors or wells for image export. Remember, only the selected images for the highlighted time point will be exported. In Figure 8-98 only Image 1 in well A1 has been selected.

If multiple images were obtained per well or sector, select which images to export. If the images are exported in bulk, then the same image will be exported for every selected well or sector. In Figure 8-98 three images were obtained per well, so it is possible to export a maximum of 3 images per well. However, in this same figure only one image (image 1) has been selected for export.

Next, use the browse button to select the target file location.

Select the Sequence Type. Recall that images can be exported as movies or images. In this case, only images are desired so highlight Set of Individual Images in the Sequence Types drop-down menu. The movie options will still be

available in this menu, but if you try to export single images as a movie, you will get an error message.

Choose Image Type:

- Phase Contrast using Current Display Settings (JPEG)
- Phase Contrast Original (JPEG)
- Phase Contrast Original (8-bit TIFF)

Enter the desired name into the Name Prefix box. The prefix will be automatically assigned additional time and area information that will appear in the Example box below.

Select Export. The Export button will display the total number of images (files) to be exported.

Time Range Image Export

Time Range Image Export is similar to Single Time Image Export, and a review of the Single Image export instructions will be useful.

Select Time Range instead of Single Time in the drop-down menu adjacent to the Time Tree.

Use the mouse or the arrow buttons to select the scan times for export. All images including and between the selected times will be exported. It is NOT possible to select non-consecutive times for export.

Select the desired wells/sectors and images.

Use the Browse button to choose the destination file.

Choose Sequence Type: Set of Individual Images.

Select the desired Image Type.

Enter a Name Prefix.

Click the Export button.

Export Image Set (Multiple Images) with Customization

Following the same setup guidelines discussed in the previous section [**Export Image Set (Multiple Images)**], it is possible to customize the set of exported images by customizing the image cropping and scaling and/or adding legend and timestamp information to each image. To accomplish this, the Sequence Type must be set to “Image set with customization.” Once this Sequence Type is selected, click on “Image Designer (Crop, Scale, Legend, etc)…” above the “Export” button at the bottom right of the “Movie/Image Set Export” window. A new window appears that is virtually identical to the Export Designer shown in Figure 8-97. As discussed in that section, the designer is used to interactively crop and scale the image and to add a legend or timestamp to each image if desired. There is an important preview tool found in the Export Designer, and we *strongly* recommend that the designer and previewer be used prior to launching a full image-set export with customization.

Export Movies Overview

A single movie can be created and exported or multiple movies can be created and exported in bulk. Because a movie by definition is comprised of a minimum of two images at consecutive time points, movies CANNOT be generated if the Single Time option is selected in the pull-down menu adjacent to the Time Tree.

Movies can be created in one of 2 common formats (Sequence Type):

- Windows Media Video
- Windows AVI Video

Similar to image export, movies can be exported using the Current Display Settings or the Phase Contrast Original settings.

Similar to export of single images, scan patterns with an excess of 9 images per well/sector require selection of a single image per well/sector or all images simultaneously.

Slideshow Mode

As a quick alternative to creating a movie, click the Slideshow button below the time tree in Vessel View to scroll through time points of the selected image without exiting Vessel View. While in Slideshow mode, you can switch to any image in the vessel by clicking on its location at the top of the window. Also, switch to the Graph/Export tab to view changes in any selected metric over time.

To change the speed at which IncuCyte™ cycles through images, click on the Preferences Menu at the top of the main IncuCyte™ screen.

Movie Export

To export movies follow the steps indicated below.

Select Time Range in the drop-down menu adjacent to the Time Tree in the Movie/Image Set Window. Choose movie start and end times. The movie will include all times including and between the selected start and end times.

Choose the wells or sectors and the desired image numbers (as described for image export). It is possible to simultaneously export movies for all images from all wells/sectors at the same time. Choose a target folder.

Select the Sequence Type.

Select Image Type Current or Phase Contrast Original settings (see Export Images Overview, pg 142).

Select a Name Prefix. As discussed in Export Image Set (Multiple Images), page 145, the Example box will be automatically populated.

If movie Sequence Type “Windows Media Video” (MWV9) or “Windows Video AVI” is selected, a button with the text “Movie Designer (Crop, Scale, Legend, etc)…” appears above the “Export” button at the bottom right of the “Movie/Image Set Export” window. See Figure 8-101. Click the “Movie Designer” button and a new window appears that is virtually identical to the Export Designer shown in Figure 8-97 of the “Export Current Image (Single Image)” section. As discussed

in that section, the designer is used to interactively crop and scale movie frames and to add a legend or timestamp to each image if desired. If a Timestamp is added, then a timestamp that displays the corresponding time (days, hours and minutes) will appear on each frame of the movie. If Show Legend is selected, a scale bar and field of view dimensions will appear in the exported image or movie as they do in Vessel View. In addition to these customization options, there is also a frames-per-second parameter that can be adjusted from a minimum of 1 to a maximum of 30 frames per second. When the mouse is placed over this control, a thumbnail preview of the movie at the selected frame rate is shown as an aid.

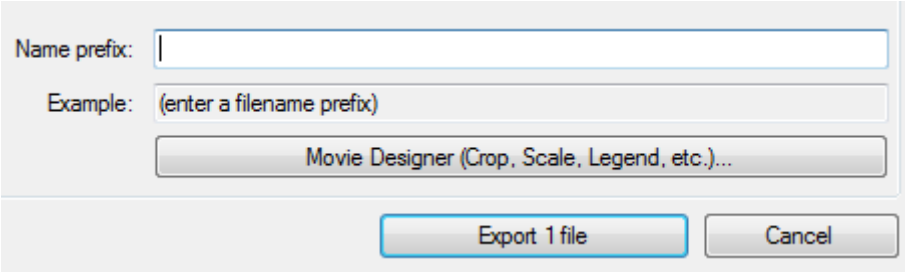


Figure 8-101. Detail of the Movie Designer option in Movie/Image Set Export

As mentioned when discussing the Export Design window, perhaps the most important tool found in the Export Designer is launched via the “Output Preview” button in the lower left corner of the Export Designer. We *strongly* encourage you to preview your customization settings prior to export! The preview allows you to view a still movie frame at the exported size as well as in a full screen mode that fills your monitor. This is often important when considering the size at which the final movie will be played. Careful consideration should be given to the final size of each movie frame, which will have a huge impact (along with the number of frames) on the ultimate size of the output movie file. Once you are satisfied with the customization, click “OK” to exit the Export Designer.

When all the parameters have been chosen, press the Export button. The Export button will indicate that number of movies (files) that will be exported under the selected conditions. In Figure 8-101 only a single movie has been selected for export.

A new window will appear indicating the status of movie generation. Multiple movies will be exported one immediately after the next until all movies have been exported and compressed.

FLR

8.7.7 Export Images and Movies FLR

Export Images

The steps required to select and export fluorescent images are the same as those required for non-fluorescent images. However, some additional Image

Types are available in the case of fluorescently scanned vessels. A summary of all available image types is listed below:

Blended Composite Using Current Display Settings (JPEG)

Fluorescence Using Current Display Settings (JPEG)

Fluorescence Uncalibrated (16-bit grayscale source PNG)

Fluorescence Uncalibrated (16-bit lossless TIFF) *

Fluorescence Calibrated (32-bit raw floating point values) *

Phase Contrast using Current Display Settings (JPEG)

Phase Contrast Original (JPEG)

Phase Contrast (8-bit TIFF)

The two image types identified with an asterisk might not be as common as the other types, but they should be interpreted by most image processing software packages. Users who wish to export images for presentation applications such as MS Office should avoid the less common formats.

Two additional image formats are available that are exclusively compatible with MetaMorph software:

- MetaMorph Single/Multi-plane Meta-Series TIFF
- MetaMorph ND File Sequence

One thing to keep in mind when generating a sequence of fluorescent images is that the fluorescence intensity of the objects and/or background can change over time. If the User does not want these changes to be reflected in the images, then the Auto-Scale Always check box should be selected prior to exporting a fluorescent image set. See Always Autoscale, page 203. To apply the Auto-Scale Always settings to exported images, the User MUST select either:

Blended Composite using CURRENT DISPLAY SETTINGS or

Fluorescence using CURRENT DISPLAY SETTINGS

NOTE: When using Fast Fluorescence, fluorescent images are smaller than normal (half-size in both dimensions) and any exported images or movies will display the resulting resolution.

Export Images from Processed Analysis Jobs

Additional image types will be available for images that have been processed. See Section 9.3, IncuCyte™ FLR - Specific Software Applications, page 204. These image types can only be exported from open Analysis Jobs.

Object Counting

Object Counting Using Current Display Settings (8-bit TIFF)

Object Counting Segmentation Mask (8-bit TIFF)

Object Counting Labeled Mask (16-bit lossless TIFF) *

Note that lossless TIFF is a less commonly used format.

Angiogenesis

Angiogenesis Using Current Display Settings (8-bit TIFF)

Angiogenesis Tube Segmentation Mask (8-bit TIFF)

Angiogenesis Tube Skeleton Mask (8-bit TIFF)

Angiogenesis Labeled Mask (16-bit lossless TIFF) *

Export Movies

The Sequence Types available for exporting fluorescent movies are the same as those for non-fluorescent movies.

One thing to keep in mind when generating fluorescent movies is that the fluorescence intensity of the objects and/or background can change over time. If the User does not want these changes to be reflected in the movie(s), then the Auto-Scale Always check box should be selected prior to exporting fluorescent movies. See Always Autoscale, page 203. After selecting the appropriate Sequence Type, select the Image Type.

Fewer image types are available for movie export than for image export, and the descriptions are fairly self-explanatory. Some additional image types are available for processed Analysis Jobs.

8.7.8 Search

Completed scans can be searched by using the Search function. See Section 8.7.9 (page 153).

8.7.9 The Scans Pull-Down Menu

The Scans pull-down menu at the very top of the page is only active in the View Completed Scans screen, See Figure 8-102. Each function within the menu is listed in addition to the corresponding key presses that can used to access the function directly. At the Administrator level, all functions are available; however, at the User/Guest levels, the Next and End Scan options are inactivated. The functions available are as follows:

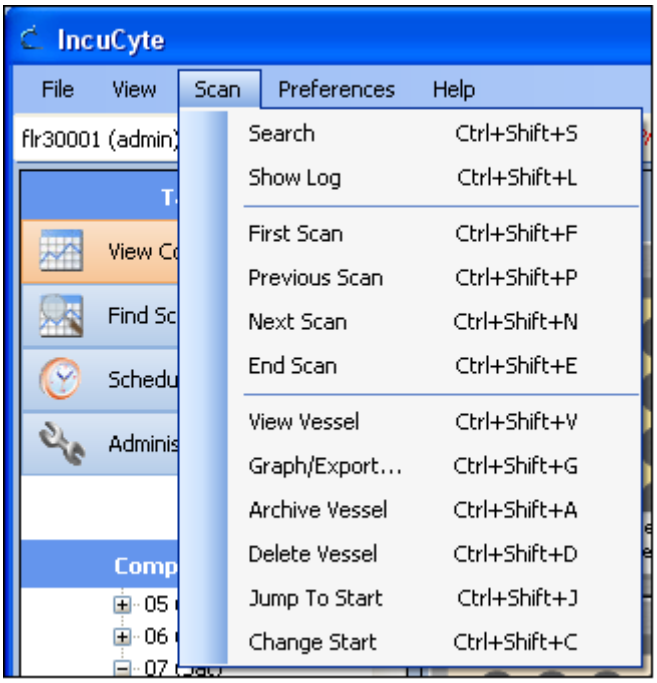


Figure 8-102. The Scans Pull-Down Menu

Search

Completed scans can be searched in one of two ways.

- Using the Search box at the top, right-hand corner of the screen.
- Using the Search option under the Scans pull-down menu.

The Scans pull-down menu offers a more comprehensive selection of search criteria than the Search box in the corner of the screen. See Figure 8-103. Search by Label, Cell Type, Passage, User Name or Notes. The Search option is NOT available for archived data.

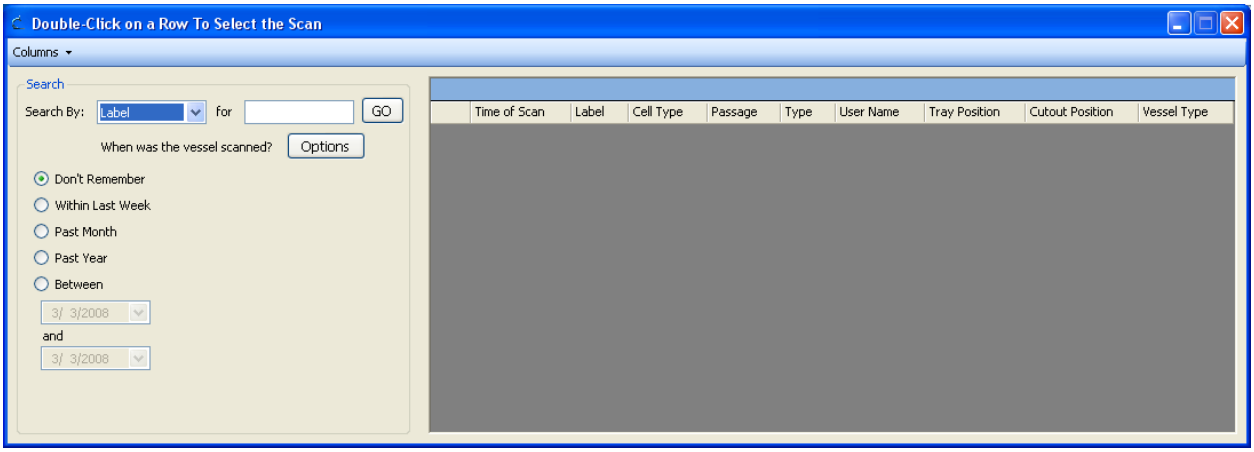


Figure 8-103. The Search Window

Select the Options button to narrow the time-frame of the search. If you know your scan occurred very recently, select the radio button for Within Last Week. If you don't recall when the scan took place, select Don't Remember. Or, if you know the scans occurred within a specific time frame, use the Between option to narrow your search to within specific dates. You CANNOT search within a single day.

Finally, the search function is not case sensitive.

Show Log

Selecting Show Log will bring up a new window displaying the Device log entries for that day ONLY.

First Scan

The First Scan option brings up the View Completed Scans screen for the very first scan recorded in the IncuCyte™.

Previous Scan

This selection brings up the View Completed Scans screen for the scan immediately previous to the one currently being displayed.

Next Scan

Selecting Next Scan brings up the View Completed Scans screen for the scan immediately following the one being currently displayed.

End Scan

End Scan brings up the very last scan recorded in the IncuCyte™.

View Vessel

Choosing View Vessel will open the Vessel View window for whichever vessel is currently selected. Vessel View is fully discussed in Section 8.7.3, Vessel View Overview, page 131.

Graph/Export

Graph/Export data associated with the selected vessel. See Section 8.10, Preparing to Graph, page 165 and Section 8.10.4, Export Metrics, page 172.

Archive Vessel

Archive an individual vessel using this option. See Section 8.6.7 (page 114).

Restore from Archive

Under some conditions, scans that were previously archived can be restored from their archive location back onto the IncuCyte™ controller. After scans have been restored, they appear on the controller as they did prior to archiving, and they do not need to be opened separately by browsing to an archive. This function is available with some limitations:

The Restore Archive function is only available on IncuCytes™ equipped with a 2009A or later software release.

Archived scans can only be restored if they were performed and archived using an IncuCyte equipped with the 2009A or later software release.

The archive that is to be restored must be derived from the same controller from which was originally archived.

Restoring archives is only recommended until 2 circumstances:

Searching Vessels (archives cannot be searched)

Running Analysis Jobs. See Manipulation of Archived Data FLR, 117.

To restore an archive, browse to the archive location and then select the scans to be restored.

Delete Vessel

Delete the selected vessel. See Delete Vessels, page 116.

Jump to Start

Select Jump to Start to jump to the very first scan of the selected vessel. This is a very useful option.

Change Start

What if you forget to check the New box when you introduce a new vessel into the scan schedule? Use the Change Start option to reset the first scan time.

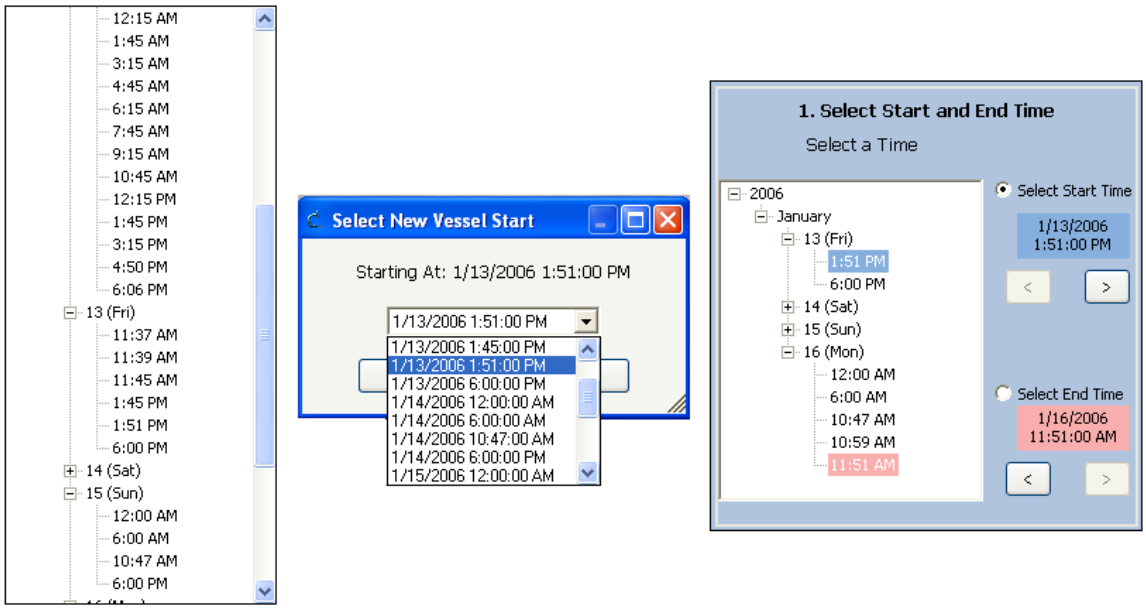


Figure 8-104. Changing the Start Time. Completed Scans are visible to the far left.

To change the start time of a vessel, follow these steps:

Select the desired vessel in the View Completed Scans screen. Then, select Change Start from the Scans pull-down menu. The Select New Vessel Start window will open. This window will list all scan times for the selected vessel plus those of the same vessel type in that same position immediately before.

Look at Figure 8-104. A new start time is being set for that vessel. The current first scan time (start time) for that vessel is January 13th, 1:51pm. This is the first time listed in the graphing Time-Tree (the Select Start and End Time window to the far right), and it is highlighted in the Select New Vessel Start window (to the immediate left). However, scans were obtained for the same vessel type immediately before 1:51pm (e.g. 1:45pm). These scans are visible in the Completed Scans Time Tree to the left and also in the Select New Vessel Start pull-down menu.

To change the first start time to 1:45pm, use the mouse to select 1:45pm in the Select New Vessel Start window.

Select OK.

The start time for that vessel will be changed to 1:45pm. Open the graphing Time Tree for the vessel. The first time listed will be 1:45pm.

EX

Change Start EX

The Change Start function is NOT available for the IncuCyte™ EX .

8.7.10 The Preferences Window

Select Preferences from the top of the View Completed Scans screen.

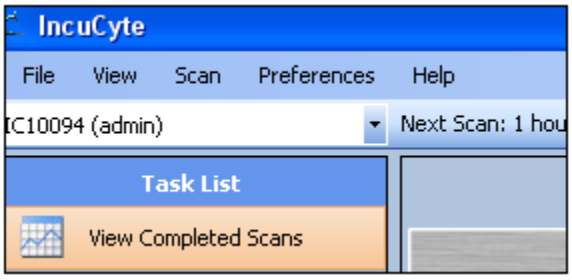


Figure 8-105. Select Preferences from the top of the screen (next to Help).

Selecting preferences will open the Preferences Window. Only the Image Drag and Drop option will be discussed here. For information on Outlier Removal and Graph Drag and Drop Auto Alignment, see Preferences: Outlier Removal and Drag and Drop Auto Alignment (page 184). For information on dragging and dropping graphs in general, see Dragging Graphs, page 181. For more information on slideshow viewing, see Slideshow Mode, page 141.

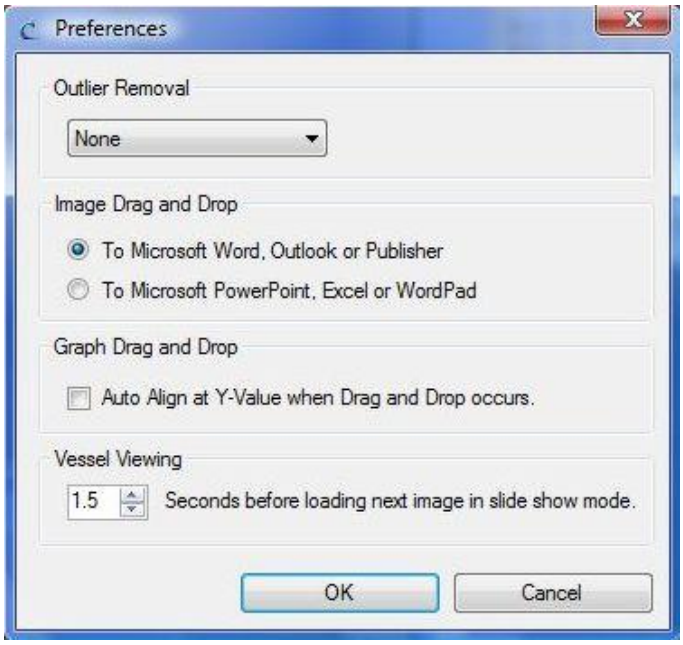


Figure 8-106. The Preferences Window

A variety of images can be dragged and dropped from the IncuCyte™ software into other programs. The following images can be dragged and dropped from the following screens:

Vessel View Window:

- The Selected Vessel image
- The large image
- The Metrics

NOTE: It is only possible to drag the vessel in the Selected Vessel box. It is NOT possible to drag individual vessel images from within a tray.

View Completed Scans Screen:

- Any tray
- The selected vessel in the Selected Vessel box
- The Metrics and image in the Selected Vessel box

Find Scanned Vessels Screen

- The selected vessel in the Selected Vessel box
- The Metrics and image in the Selected Vessel box

Select the appropriate option in the Preferences Window based on the intended destination of the image Drag and Drop. See Figure 8-107. Whichever option is selected will apply to all drag and drops until the option is changed.

NOTE: T asks) will have a backgrou

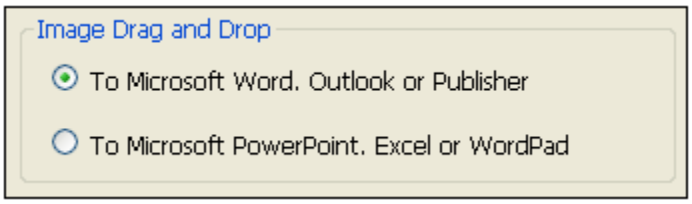


Figure 8-107. Image Drag and Drop Destination

8.8 Find Scanned Vessels

Select the Find Scanned Vessels task bar to open the corresponding software screen. Use this screen to search for previously scanned vessels. See Figure 8-108.

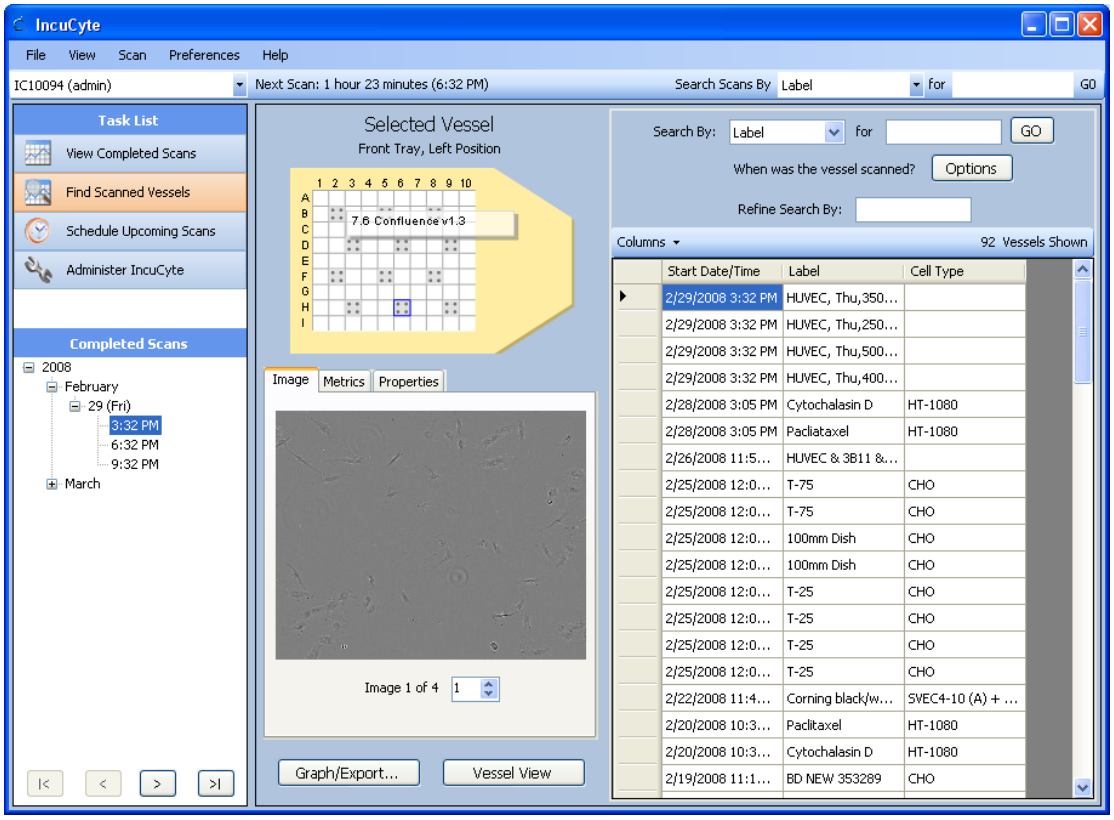


Figure 8-108. The Find Scanned Vessels Screen.

The Find Scanned Vessels screen contains 4 components:

- The Search box
- The Search Results (displayed below the Search box)
- The Selected Vessel box

- The Time Tree for the selected vessel

8.8.1 Searching

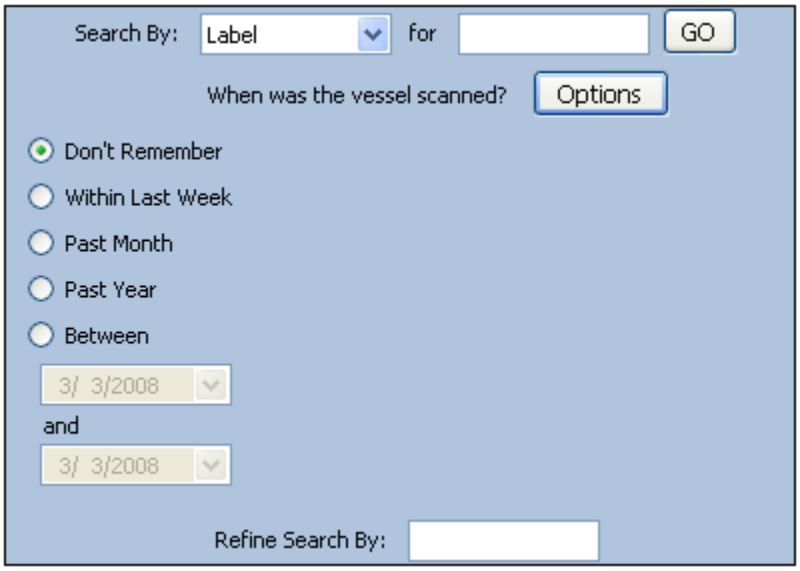


Figure 8-109. The Search Box

Press the Options button to refine the time frame of your search. In Figure 8-108, the Options button has not been selected. Figure 8-109 shows a close-up of the expanded search opportunities that are enabled following selection of the Options button. Choose either a general time frame (e.g. Past Month) or use the Between radio button to define more specific temporal boundaries.

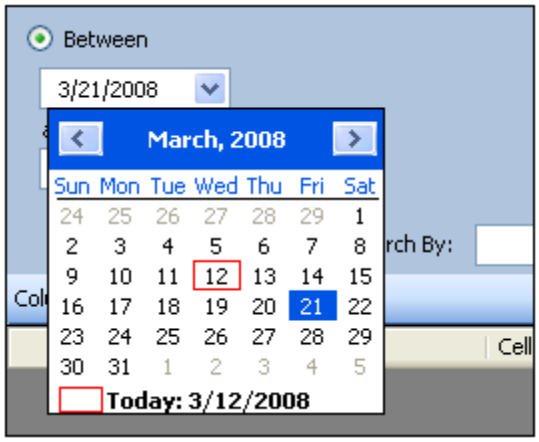


Figure 8-110. Selecting the Precise Time Frame of the Search

Search Terms

Select the Search criteria by Parameter (Label, Cell Type or Passage) and enter one or more search terms into the For box.

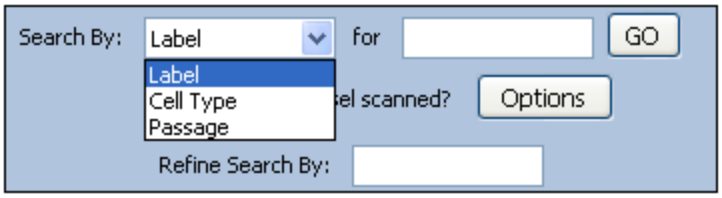


Figure 8-111. Search Parameters

ONLY Label, Cell Type and Passage can be searched using this function. To search other scan-related terms (such as those related to Properties, Passage, etc) use the Search window at the top of the View Completed Scans screen (see Section 8.7.9, page 153). Search terms can include numbers, letters and special characters. Press the GO button to initiate the search.

The Search function operates as follows:

- One or more individual terms entered into the For box will find any scan that contains any one of those terms, in the specified "Search By" field.
- Enclosing two or more terms in quotes will display any scan that contains those exact terms in that exact order.
- The * symbol can used as a "wild card" in the middle of a Search term. For instance the Search S*N will return the results for Scan or Super Scan.

The terms entered into the Search Box act as a filter. In other words, whatever terms are entered for searching are used to exclude entries that do NOT contain the relevant terms. Therefore, if no terms have been entered, the search results will display all scans that have occurred during the selected time frame (see Figure 8-108).

NOTE: Searches are NOT case-sensitive.

Example Searches:

- Search Label: **test 96**. The results include all scans with labels that contain EITHER **test** or **96** (7 vessels). See Figure 8-112.

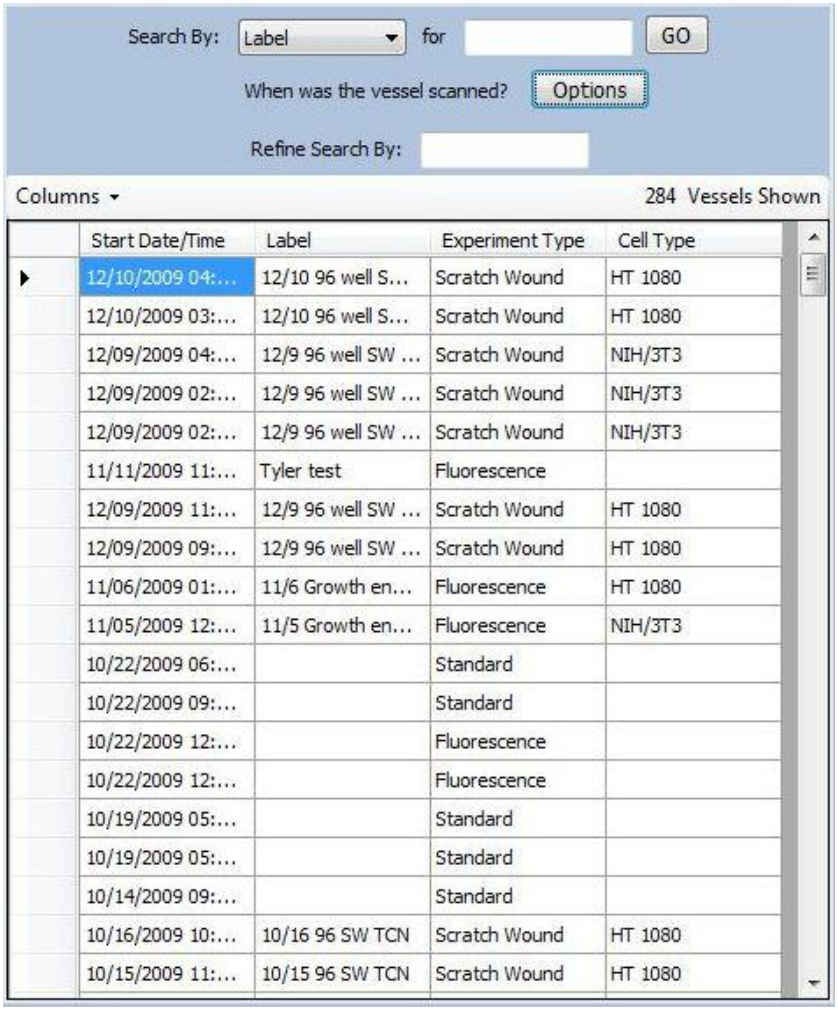

Search By: Label for GO
When was the vessel scanned? Options
Refine Search By:
Columns
284 Vessels Shown
Start Date/Time | Label | Experiment Type | Cell Type
12/10/2009 04:... | 12/10 96 well S... | Scratch Wound | HT 1080
12/10/2009 03:... | 12/10 96 well S... | Scratch Wound | HT 1080
12/09/2009 04:... | 12/9 96 well SW ... | Scratch Wound | NIH/3T3
12/09/2009 02:... | 12/9 96 well SW ... | Scratch Wound | NIH/3T3
12/09/2009 02:... | 12/9 96 well SW ... | Scratch Wound | NIH/3T3
11/11/2009 11:... | Tyler test | Fluorescence |
12/09/2009 11:... | 12/9 96 well SW ... | Scratch Wound | HT 1080
12/09/2009 09:... | 12/9 96 well SW ... | Scratch Wound | HT 1080
11/06/2009 01:... | 11/6 Growth en... | Fluorescence | HT 1080
11/05/2009 12:... | 11/5 Growth en... | Fluorescence | NIH/3T3
10/22/2009 06:... | | Standard |
10/22/2009 09:... | | Standard |
10/22/2009 12:... | | Fluorescence |
10/22/2009 12:... | | Fluorescence |
10/19/2009 05:... | | Standard |
10/19/2009 05:... | | Standard |
10/14/2009 09:... | | Standard |
10/16/2009 10:... | 10/16 96 SW TCN | Scratch Wound | HT 1080
10/15/2009 11:... | 10/15 96 SW TCN | Scratch Wound | HT 1080

Figure 8-112. Search Label for test 96

- Now, Search Label **"test 96"**. See Figure 8-113. By enclosing the terms in quotation marks, the search looks for all scan labels that include the terms **test** AND **96** together in that order (for the same vessel). There were no results using this search string.

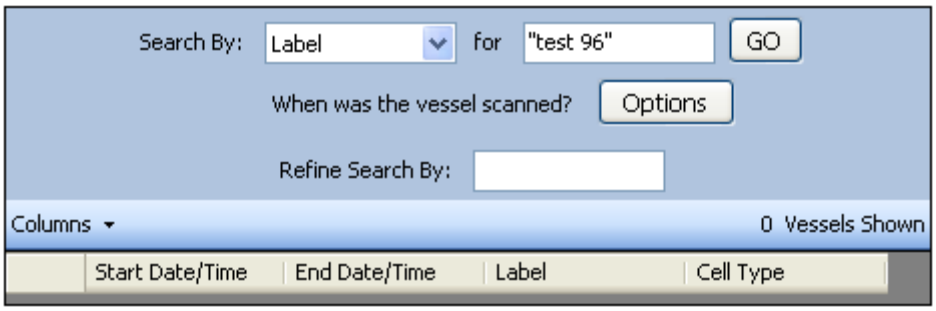

Search By: Label for "test 96" GO
When was the vessel scanned? Options
Refine Search By:
Columns
0 Vessels Shown
Start Date/Time | End Date/Time | Label | Cell Type

Figure 8-113. Search Label for "test 96"

- Finally, search Label t*t. See Figure 8-114.

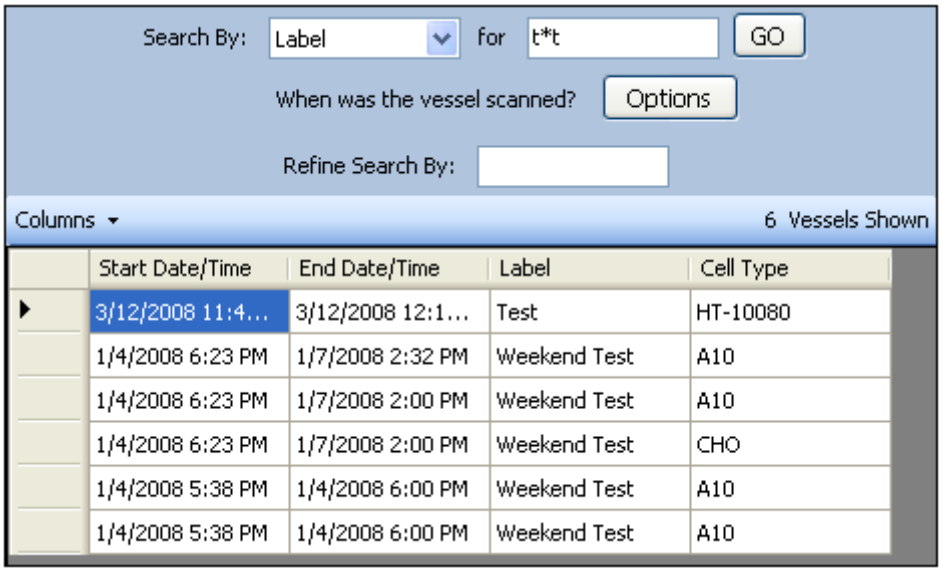


Figure 8-114. Search Label for t*t

Refine Search By

Search results that were obtained using the Search By function can be filtered once more using the Refine Search By box. The Refine Search function performs a follow-up search on the results of the "coarse" Search By function. Note that the Refine Search function can simultaneously filter the results within both the Label and the Cell Type columns. For example, Refine the search in Figure 8-114 by the term Weekend. The Refine Search results are displayed in Figure 8-115. Now, the Vessel with the label Test has been eliminated, and only 5 vessels are displayed instead of 6.

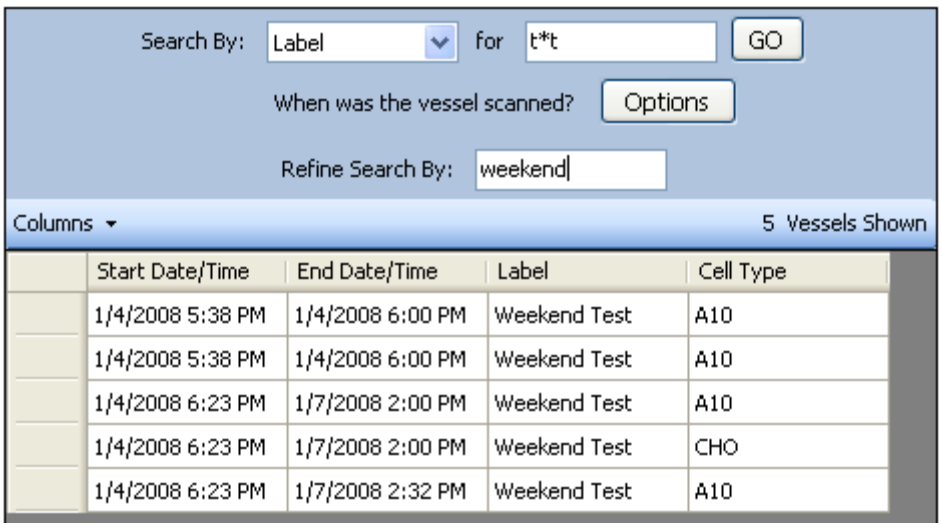


Figure 8-115. Refine Search by weekend

In summary, the rules for the Refine Search By function are as follows:

- Only a single term can be searched.
- It is NOT possible to use the * symbol as a wild-card.
- The Refine Search function ONLY filters the results DISPLAYED as a result of the initial, coarse search.
- The Refine Search function ONLY filters the Label and Cell Type columns. It does not apply to the Start Date/Time, End Date/Time and Passage columns. To search according to dates or passage, use the Passage option in the coarse search and/or define the specific dates of interest.

Search Results

The search results will include any vessel that was part of a scan containing the selected search terms and occurring during the prescribed time span. Each line in the results section represents a single vessel. The Start Date/Time and the End Date/Time for each vessel is indicated, along with the vessel label, cell type, and experiment type. Selecting/highlighting one of the vessels will display the vessel in the Selected Vessel box to the left of the search results. All available scan times/dates for that vessel will be displayed in the Time Tree.

The Vessel Box will automatically display the image for the very first scan of the selected vessel. Scroll through the Time Tree to view scan results as in the View Completed Scans screen.

Displayed Columns

The number of columns that are displayed in the Results area can be adjusted. Click the Columns pull-down menu above the search results to view display options. By default, the Start Date/Time, Label, Experiment Type and Cell Type will be displayed. The remaining two parameters (End Date/Time and Passage) can be displayed if they are selected under the Columns pull-down menu. Alternatively, the columns for any of the currently selected parameters can be unselected. Remember that when a Search is performed, only columns that are selected for display can be searched. So, if you want to search by Cell Type, be sure that the Cell Type column is being displayed BEFORE starting the search.

When all 5 columns are selected, use the slide bar at the bottom of the screen to visualize them all.

8.8.2 The Selected Vessel Box

The Selected Vessel Box in the Find Scanned Vessels screen is no different from the same box in the View Completed Scans screen. Highlight vessels in the search results to display the corresponding Selected Vessel Box.

EX

8.9 **Find Scanned Vessels EX**

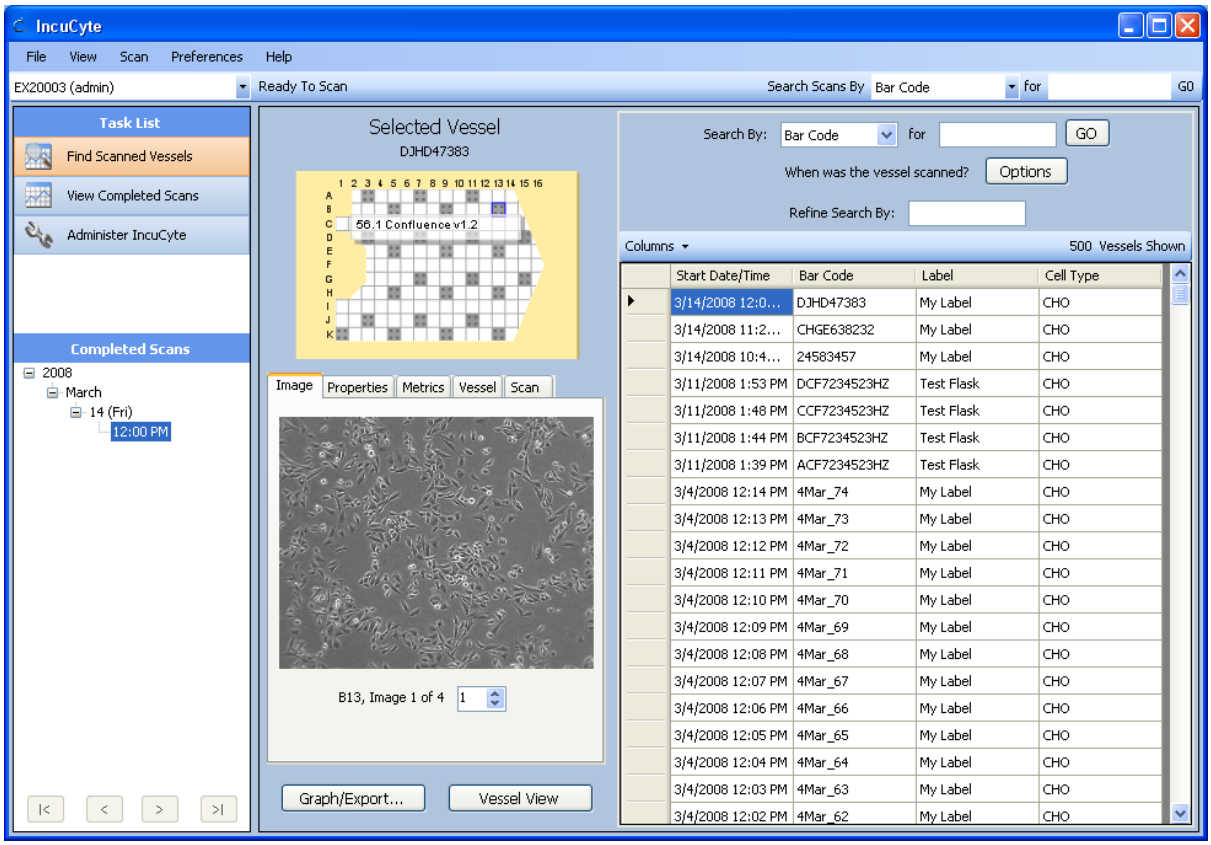

IncuCyte
File View Scan Preferences Help
EX20003 (admin)
Ready To Scan
Search Scans By Bar Code for GO
Task List
Find Scanned Vessels
View Completed Scans
Administer IncuCyte
Completed Scans
2008
March
14 (Fri)
12:00 PM
Selected Vessel
DJHD47383
56.1 Confluence v1.2
Image Properties Metrics Vessel Scan
B13, Image 1 of 4
Graph/Export...
Vessel View
Search By: Bar Code for GO
When was the vessel scanned? Options
Refine Search By:
Columns
500 Vessels Shown
Start Date/Time Bar Code Label Cell Type
3/14/2008 12:0... DJHD47383 My Label CHO
3/14/2008 11:2... CHGE638232 My Label CHO
3/14/2008 10:4... 24583457 My Label CHO
3/11/2008 1:53 PM DCF7234523HZ Test Flask CHO
3/11/2008 1:48 PM CCF7234523HZ Test Flask CHO
3/11/2008 1:44 PM BCF7234523HZ Test Flask CHO
3/11/2008 1:39 PM ACF7234523HZ Test Flask CHO
3/4/2008 12:14 PM 4Mar_74 My Label CHO
3/4/2008 12:13 PM 4Mar_73 My Label CHO
3/4/2008 12:12 PM 4Mar_72 My Label CHO
3/4/2008 12:11 PM 4Mar_71 My Label CHO
3/4/2008 12:10 PM 4Mar_70 My Label CHO
3/4/2008 12:09 PM 4Mar_69 My Label CHO
3/4/2008 12:08 PM 4Mar_68 My Label CHO
3/4/2008 12:07 PM 4Mar_67 My Label CHO
3/4/2008 12:06 PM 4Mar_66 My Label CHO
3/4/2008 12:05 PM 4Mar_65 My Label CHO
3/4/2008 12:04 PM 4Mar_64 My Label CHO
3/4/2008 12:03 PM 4Mar_63 My Label CHO
3/4/2008 12:02 PM 4Mar_62 My Label CHO

Figure 8-116. Find Scanned Vessels Screen in the EX

When the IncuCyte™ EX software is launched, it opens by default to the Find Scanned Vessels Screen. See Figure 8-116. In most respects, the Find Scanned Vessels screen in the EX functions as previously described in Section 8.8: Find Scanned Vessels, starting on page 158. However, there are some important differences.

Because the EX identifies vessels by barcode, the Search function is expanded to include the Bar Code parameter. See Figure 8-116. Thus, the default searchable parameters available in the Search By box are:

Bar Code

Label

Cell Type

Passage

In the EX, the parameters available in the Search By pull-down menu also include the available Custom Vessel Fields (see Section 8.5.6, page 75 for more information on Custom Vessel Fields). In Figure 8-117, the first Custom Vessel Field has been assigned the name Seeding Date, and Seeding Date is now available in the Search By pull-down

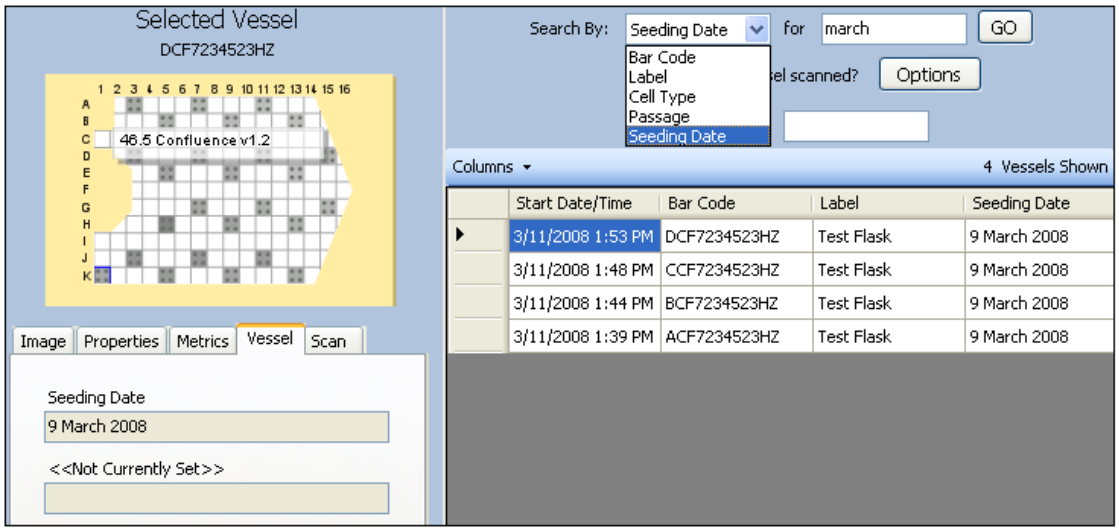


Figure 8-117. Adding Custom Vessel Fields in the EX

menu. A Search was performed using Search By Seeding Date For March, and the results are displayed in Figure 8-117. Note that an additional column has been created to display the results of the Seeding Date Search. However, even

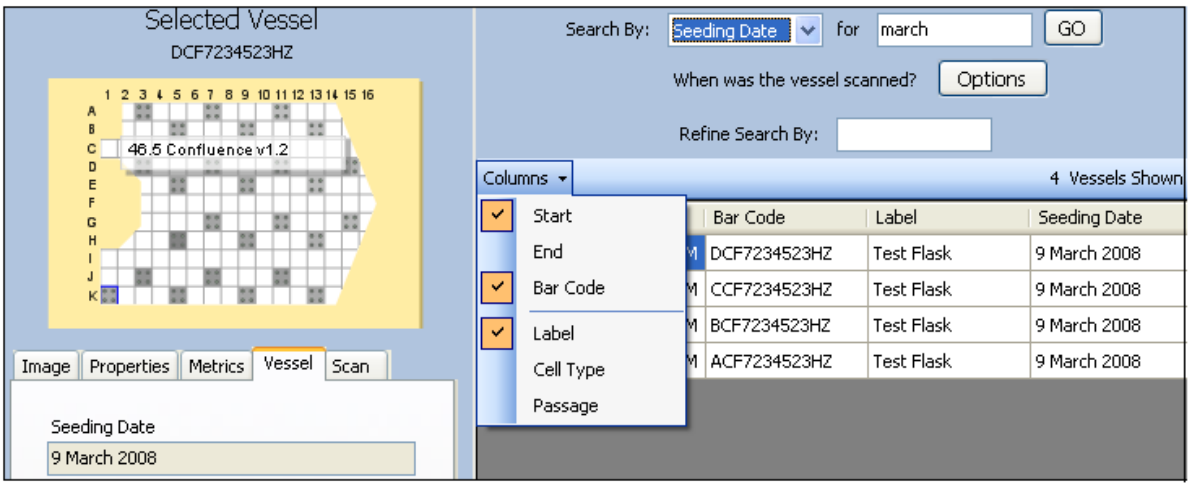


Figure 8-118. Columns Pull-Down Menu Remains the Same (EX)

though and an extra column exists to display the results of the Seeding Date search, note that the Seeding Date column is NOT available in the Columns pull-down menu. See Figure 8-118. Additional Custom Vessel Parameters will only be displayed as columns in the results section if they were actively searched.

8.10 Preparing to Graph

The IncuCyte™ graphing feature can be accessed in one of 4 ways:

- Selecting the Graph/Export button in the Selected Vessel box in the Completed Scans screen. This will open the Graphing Box.
- Selecting the Graph/Export tab in the Vessel View window (See Graph/Export Tab, page 136).
- Choosing the Graph option from the Scan pull-down menu (Section 8.7.9, page 152).
- Right-clicking on a selected vessel in the View Completed Scans screen (See Tasks Available by Right-Clicking on a Selected Vessel, page 131).

Note that graphing from the Vessel View window offers some additional options that are not available via the View Completed Scans screen. To access graphing functions through the Vessel View window, select the Vessel View Graph/Export tab (see Figure 8-120) and then press the Graph/Export button. Pressing this button will open a new window. See Figure 8-122, page 170.

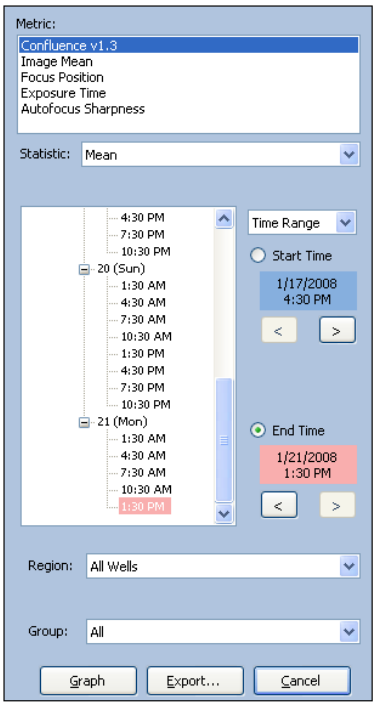


Figure 8-119. The Graphing Box as Displayed in the View Completed Scans Screen

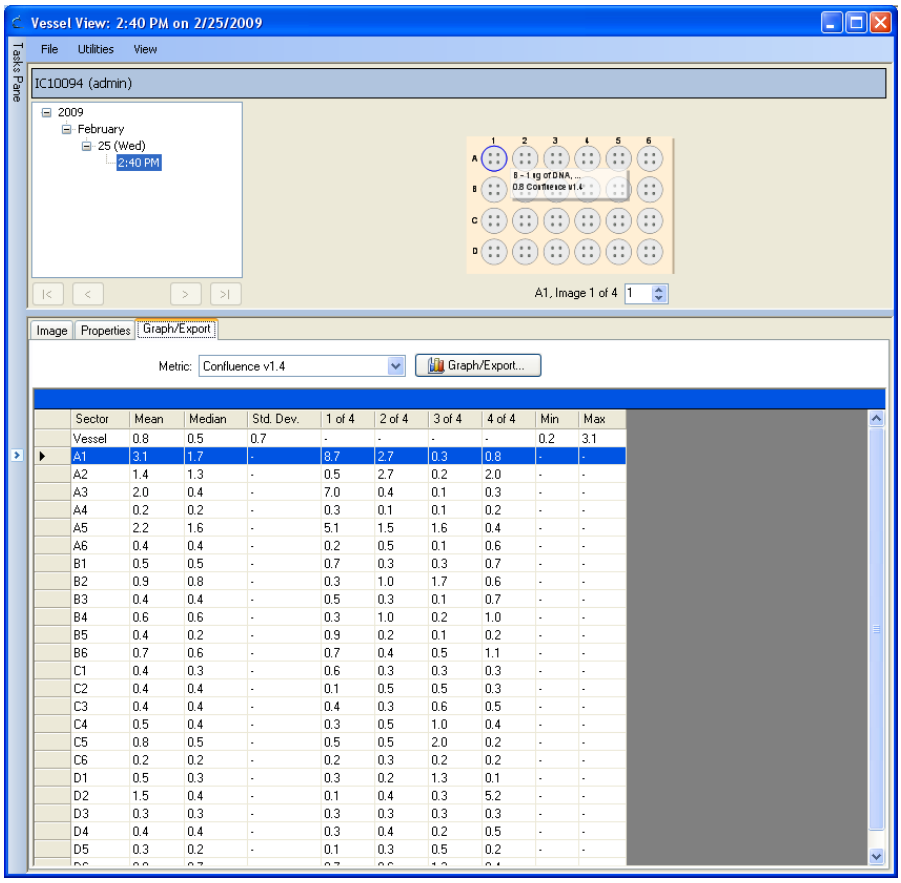


Figure 8-120. The Graph/Export Tab in the Vessel View Window

8.10.1 Prepare to Graph from the View Completed Scans Screen

The default Metric is Confluence. It is also the most commonly used Metric. The remaining Metrics: Image Mean, Focus Position, Exposure Time and Autofocus Sharpness are diagnostic tools used to confirm that the software is functioning within the specified parameters. These Metrics will not be relevant to the User under normal circumstances.

NOTE: Metrics other than Confluence are only available at the Administrator permission level.

Select Statistic

Image Mean/Median

When multiple images are available within each well, then selecting the Mean vs. Median will select the image Mean or image Median respectively for graphing. If a single well is graphed, then each point on the graph will represent the Mean or Median for all images in that well at that time.

Trace Mean/Median

When multiple wells are represented by each point on the graph (for instance, if all wells within a column are combined to generate a graph of that column), then the Median or Mean refers to the combination of the individual wells.

Summary:

1. If each point on a graph represents a single well or sector, then selecting the Mean or Median refers to the Mean or Median of all the images within that well or sector. This is the case when the data are graphed as a single well or sector.

2. If each point on a graph represents multiple wells or sectors, then selecting Mean or Median refers to the Mean or Median of all the wells/sectors that comprise that data point. Only the image MEANS of multiple wells/sectors will be combined. Therefore, selecting Mean or Median for multiple wells/sectors will generate a graph consisting of the trace Mean or Median of the image MEANS.

Select Start and End Time

The Time Range over which the data are to be graphed can be selected using the Start and End Time buttons to the right of the Time Tree. In order to graph, the Time Range option must be selected in the box above the Start Time radio button. The alternate selection, Single Time, is only applicable to data export. The default settings are the times and dates of the first and last scans in the series. The chosen Start and End times will be highlighted in blue and pink respectively. After the Start or End Time radio button is selected, the time can be changed by:

- Directly selecting a time with the mouse
- Using the arrow buttons to sequentially scroll through the times until the chosen set point has been reached

Graphing Microplates

The Microplate Graph

Users can use the Microplate Graph function to graph all wells or selected wells in a microplate format. That is, all wells are plotted individually as they would appear in a microplate (6-well through 384-well). The colors of the Microplate Graph would be directly correlative to the colors selected in the Plate Map Editor. For more information on Microplate Graphs, please refer to Section 8.10.6, page 199.

If individual graphs are desired, users can divide the plate into regions and graph on separate axes. Regions of a microplate vary from large to small, encompassing the entire plate, single columns, single rows or individual wells.

With the exception of individual wells, graphical results can be viewed as grouped or not grouped.

Regions and Grouping

Select the Region pull-down menu to display all possible microplate regions. See Figure 8-121.

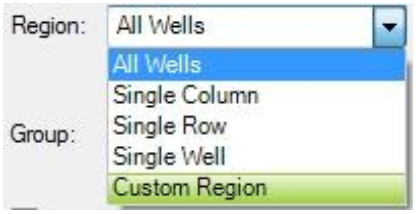


Figure 8-121. Select Region

All Wells

Select All Wells to simultaneously graph the Mean or Median for all wells within a microplate. The Region All Wells (meaning the entire plate) provides the largest number of grouping possibilities. See below:

- Group All: All the wells of the microplate will be combined into a single trace.
- Group Columns: All the wells of the microplate will be graphed as columns. For example, a 24-well plate will generate a graph of 6 columns.
- Group Rows: All the wells of the microplate will be graphed as rows. For example, a 24-well plate will generate a graph of 4 rows.
- Group None: In this case, none of the wells will be grouped. A 24-well plate will generate a graph of all 24 wells. Error bars are not available when single wells are graphed.

Single Column or Row

In this case, the only region of interest is a specific Column or Row within the microplate. Selecting one of these regions will open a new drop-down menu to facilitate further selection of the specific column or row of interest. Single Columns and Rows offer only two grouping options: All or None. Either all the wells within the selected Column or Row can be combined to generate a single trace, or none of the wells within the selected Column or Row will be combined, resulting in multiple traces (one for each well).

Single Well

No grouping options are available for graphing a single well. Scroll through the possible wells to find the well that is desired for graphing. This selection will generate a single trace for the selected well. Error bars are not available when a single well is graphed.

Single Well (96 and 384-Well Plates)

High-density microplates (microplates containing 96 or 384-well wells) have a modified selection format for graphing a single well. Because scrolling through

all 384 wells to select just the single well desired for graphing would be inconvenient, in the case of high-density plates it possible to independently select both the Row letter and the Column number.

Graphing Flasks and Dishes

Flasks and Dishes are simpler to graph than microplates. They contain only two possible regions:

- All Sectors
- Single Sector

Region All Sectors

If All Sectors are selected, the graphs can be viewed with or without error bars, and the error bars will represent the inter-sector Mean or Median as selected in the Statistic drop-down menu.

All: All the sectors within the flask will be combined into a single trace.

None: any of the sectors will be grouped. No error bars are available for graphs of single sectors.

Region Single Sector

If the Region Single Sector is selected, a new drop-down menu will appear. Choose the desired sector for graphing. Error bars are not available for graphs of a single sector.

8.10.2 Prepare to Graph From the Vessel View Window

Press the Graph/Export button in the Graph/Export tab of the Vessel View window to open a new graphing window. See Figure 8-122. In this figure, a single well, A1, has been selected. Note that it is possible to independently choose both the well letter and number (because this is a high-density plate).

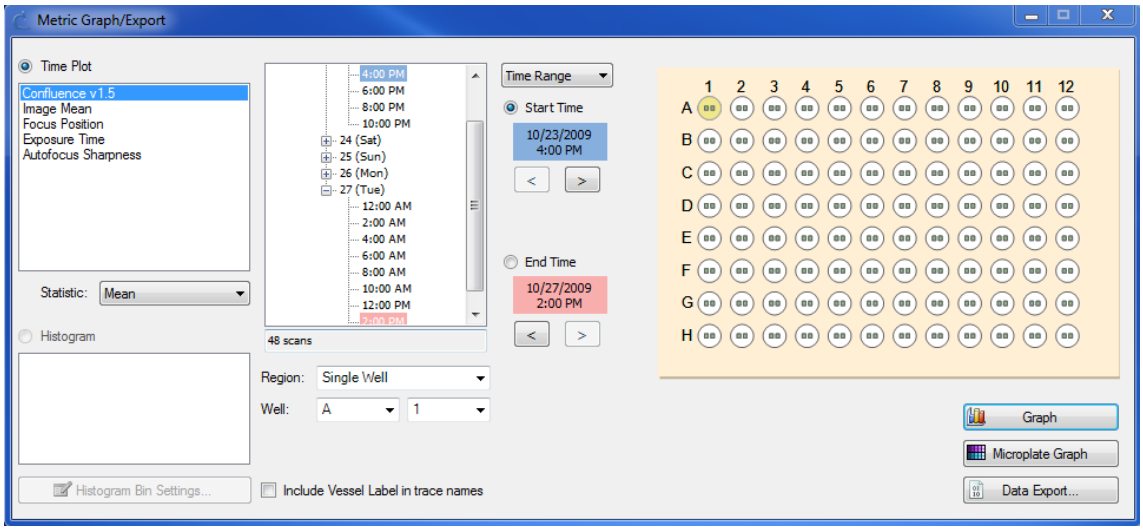


Figure 8-122. The Graphing Window from the View Completed Scans Screen

The overall Graphing features available in the Vessel View window are the same as those available using the View Completed Scans Graphing Box, but there are some additional options specific to the Vessel View.

Custom Region

The Region pull-down menu in the Vessel View window offers a Custom Region option. See Figure 8-123. When Custom Region is selected, the vessel color will change from light orange to green, thus alerting the User that this is a special

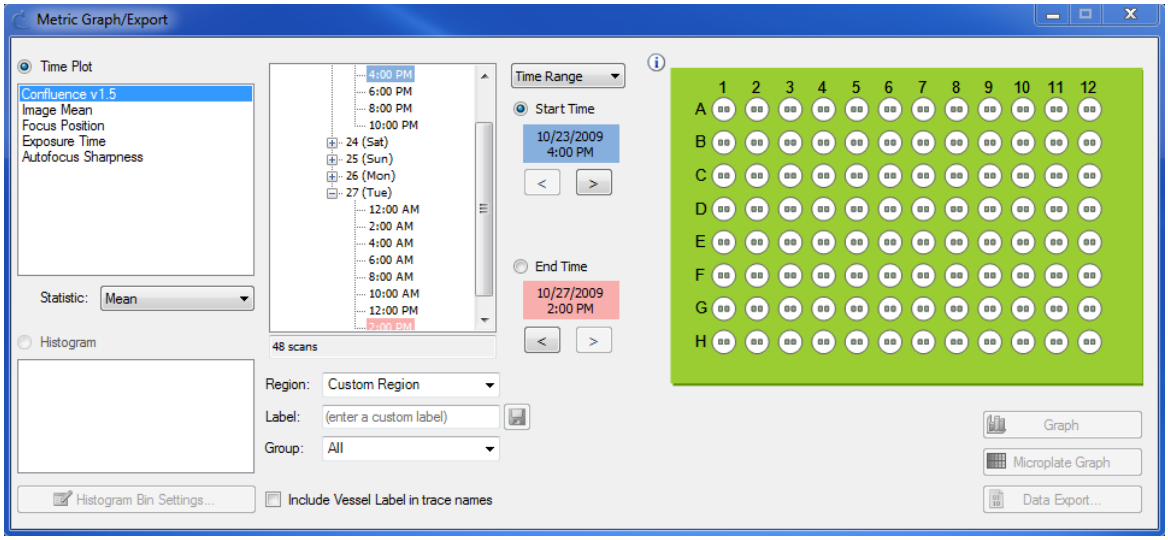


Figure 8-123. Custom Region Selected

graphing Mode. When Custom Region is selected, the User can select/deselect a subset of individual wells with the mouse as desired. Normally, when a graph is generated, it is automatically assigned a Label based upon the selected Region and Statistic. Because in this case the Region is variable, it is possible for the User to assign a custom Label. If the Label box is not populated, the default Label will simply read Custom Region accompanied by the Statistic.

To save a custom region for later use, click the Save button to the right of the Label box. The custom region will then be saved under the Region pull-down menu for the current vessel type. To delete a saved custom region, select it from the Region pull-down menu and click the "X" next to the Region box. The selected region will then appear as a Custom Region, giving the option to edit it or permanently delete it (by choosing a different saved region).

Regions from a Plate Map

If a plate map is associated with a vessel (microplates only), any Region created in the Plate Map Editor will be available for graphing. To create a plate map or assign regions, see Section 8.7.5 (page 136).

Histograms

Currently, it only possible to create Histograms when graphing Object Counting data, and the Histogram option is disabled in Figure 8-123. See Graph/Export

Histograms of Processed Jobs, page 232 for information on graphing histograms. However, it is possible that the Histogram function will be expanded in the future.

FLR

8.10.3 Prepare to Graph FLR

It is ONLY possible to graph fluorescent Metrics from the Vessel View window. Non-fluorescent Metrics (e.g. confluence) can be graphed using the Graphing Box in the View Completed Scans screen.

Some additional Metrics are available for fluorescent scans that are not available for non-fluorescent scans. Most FLR-specific Metrics are only available for Job Previews or Completed Analysis Jobs (further reading on this topic is recommended. See Section 9.3.9, page 231).

8.10.4 Export Metrics

It is possible to export the Metrics associated with a particular vessel directly to another document or to a selected file. There are a variety of options regarding the format in which the data are exported. To export vessel Metrics, select the Export button rather than the Graph button at the bottom of the graphing window. Choosing the Export button will open a new window in which details of the selected export format can be chosen. See Figure 8-124.

The scans for which data will be available for export are selected using the Time Tree in the graphing box. The default settings are the first and last scans available for the selected vessel. If a vessel was scanned 20 times, but only the data from the first 10 scans need to be exported, then use the mouse or arrow buttons to select the desired Time Range. If only the data from a single scan need to be exported, choose Single Time instead of Time Range from the drop-down menu above the Start Time radio button.

If Time Range is selected, then the data set that is exported will be a continuous set within the given time span. It is NOT possible to simultaneously export data from individual, temporally discontinuous scan times. If discrete, discontinuous data sets are desired, then each data set must be exported individually.

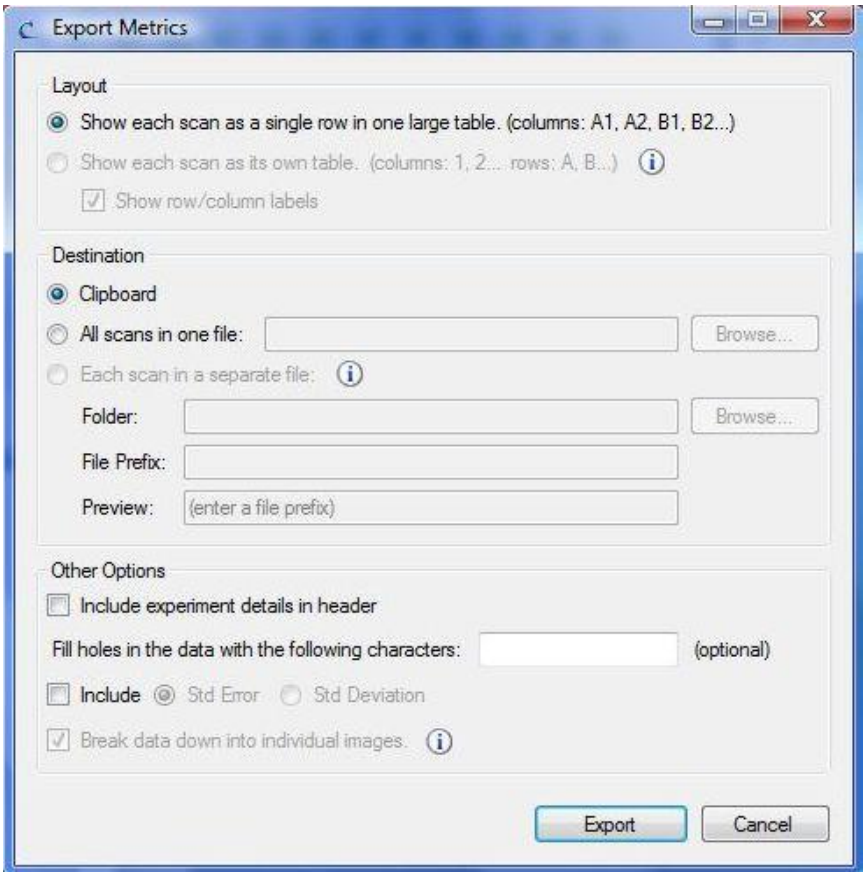


Figure 8-124. The Export Metrics Window

Note that in Figure 8-124 some of the options are inactive. Which regions of the Export Metrics window are active are determined by selections made within the Export Metrics window as well as those Region and Grouping settings made prior to opening the Export Metrics window. For example, under the Layout heading, Show Each Scan as its Own Table can only be activated if the Group option was set to None during the Graph/Export Setup (see Figure 8-119 and Figure 8-120).

The Export Metrics window is divided into 3 main sections:

- Layout
- Destination
- Other Options

Each of these sections will be discussed individually.

NOTE: Show Each Scan as its Own Table will only be an available if Group: None was selected in the graphing window.

Layout

Use this section to select the overall Layout of the exported data. The data for each scan time can be displayed within a single long row or displayed as its own table.

Show Each Scan as a Single Row

In this case, each scan time will be listed to the far left, and the data for each well will be displayed as a single row to the right. In Figure 8-125 the data are displayed as a single row. In this figure, Region: All Wells, Group: None were selected prior to opening the Export Metrics window. Note that wells from which no data were obtained are omitted.

Experiment: Corning 384

Date Time	Elapsed	Corning 384: A1	Corning 384: E1
1/17/2008 16:30	0	4.28	9.21
1/17/2008 19:30	3	5.53	10.4
1/17/2008 22:30	6	6.57	12.6
1/18/2008 1:30	9	8.18	15.3

Figure 8-125. Data as a Single Row

Show each Scan as its own Table

Alternatively, the data can be displayed as a single table, one table for each scan time. This option will only be available if Group: None was selected in the Graph/Export setup prior to pressing the Export button. If each scan is displayed as its own table, the columns labels can be displayed or not by checking the corresponding check box. If data are exported in the form of a table, then wells or sectors from which no data were obtained (because they were skipped in the scan pattern) will appear as blank spaces. See the section about Other Options, page 175 for more information on this topic.

Destination

Choose a destination for the exported data. There are two possible destinations for exported data:

- Export to Clipboard
- Export to one or more files

Export to Clipboard

Choose export to Clipboard to export the data directly to a new location such as EXCEL®, PowerPoint® or MS Word®. Simply select export to Clipboard, press the Export button and then navigate to the destination software. Then, choose Paste. The data should appear in the previously selected format.

Export Data to One or More Files

The data can be exported to a single file by selecting the radio button for All Scans in One File. For example, suppose a 96-well plate was scanned at 10 different time points. If the data are exported to a single file, when the file is opened it will contain the data for all 10 scan times.

Alternatively, the data from each time point (each scan) can be saved to a different file. In this case, select Each Scan in a Separate File.

NOTE: The option of saving Each Scan in a Separate File will only be available if the radio button for Show each Scan as its Own Table is selected in the Layout Section of the Export Metrics window.

Continuing with the same example as above, a 96-well plate that was scanned at 10 time points will generate 10 individual files, and each file will contain the data for a single time point. To save scans to separate files, browse to a destination file and then enter a file prefix. The Preview box will be populated automatically as the File Prefix is entered. Press the Export button.

Whether the data are exported in a single or multi-file format, the file(s) will be saved in text format with a ".txt" extension. Open the data as a text file, or open the file in an alternate software program and then save to that program as desired.

Other Options

Two additional options are available when exporting data:

- Include Experiment Details in the Header
- Fill Holes in the Data with the Following Characters
- Include Error Measurements
- Break Data Into Individual Images

Include Experiment Details in the Header

Choose this box to include export of the following additional details along with the data: the selected Metric (usually confluence), cell type, passage number, and any notes. If multiple files are exported, the additional details will appear in every file.

Fill Holes in the Data with the Following Characters

It is possible to set a scan pattern that does not include all the possible wells or sectors in the vessel. If the data are exported using the Layout option: Show each Scan as its Own Table, the table will have empty spaces where sectors or wells were omitted from the scan pattern. See Figure 8-126.

Include Error Measurements

Use this option to include either standard error (SE) or standard deviation (SD) for each measurement. Note that SE and SD are not available for individual images, but are calculated for the selected grouping (all, rows, columns, none). When None is selected, error measurements are available only if there are multiple images per sector.

Break Data Into Individual Images

When there are multiple images per sector, you have the option to break data down into measurements from each image.

Time Stamp:	1/17/2008 16:30	Elapsed:	0	hours
	1	2	3	4
A	4.28			
B		9.14		
C			4.59	
D				4.03
E	9.21			
F		5.08		
G			5.88	
H				4.64
I	2.85			
J		4.48		
K			7.02	
L				8.63
M	4.87			
N		7.54		
O			7.29	
P				6.5

Figure 8-126. Export of a 384-well plate without filling the "Holes."

Rather than leaving these spaces empty, it is possible to select characters to fill the spaces. In Figure 8-127, the word “BLANK” has been selected for the empty spaces.

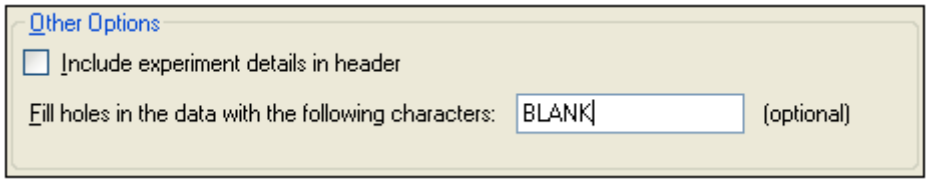


Figure 8-127. Fill Holes with the Word "BLANK"

Export the same data set, and the results will now be displayed as shown in Figure 8-128.

Time Stamp:	1/17/2008 16:30	Elapsed:	0	hours
	1	2	3	4
A	4.28	BLANK	BLANK	BLANK
B	BLANK	9.14	BLANK	BLANK
C	BLANK	BLANK	4.59	BLANK
D	BLANK	BLANK	BLANK	4.03
E	9.21	BLANK	BLANK	BLANK
F	BLANK	5.08	BLANK	BLANK
G	BLANK	BLANK	5.88	BLANK
H	BLANK	BLANK	BLANK	4.64
I	2.85	BLANK	BLANK	BLANK
J	BLANK	4.48	BLANK	BLANK
K	BLANK	BLANK	7.02	BLANK
L	BLANK	BLANK	BLANK	8.63
M	4.87	BLANK	BLANK	BLANK
N	BLANK	7.54	BLANK	BLANK
O	BLANK	BLANK	7.29	BLANK
P	BLANK	BLANK	BLANK	6.5

Figure 8-128. Empty spaces are filled with the word "BLANK."

8.10.5 Create a Graph

Create a graph

From the View Completed Scans screen with the graphing window open choose the graphing Metric (Confluence), the Start and End times, the Region and Group. See Figure 8-129. The graphing window is open to the right. Start and End times have been selected. The Metric (Confluence) is highlighted, and the Statistic: Mean, Region: All Wells, and Group: All have been selected.

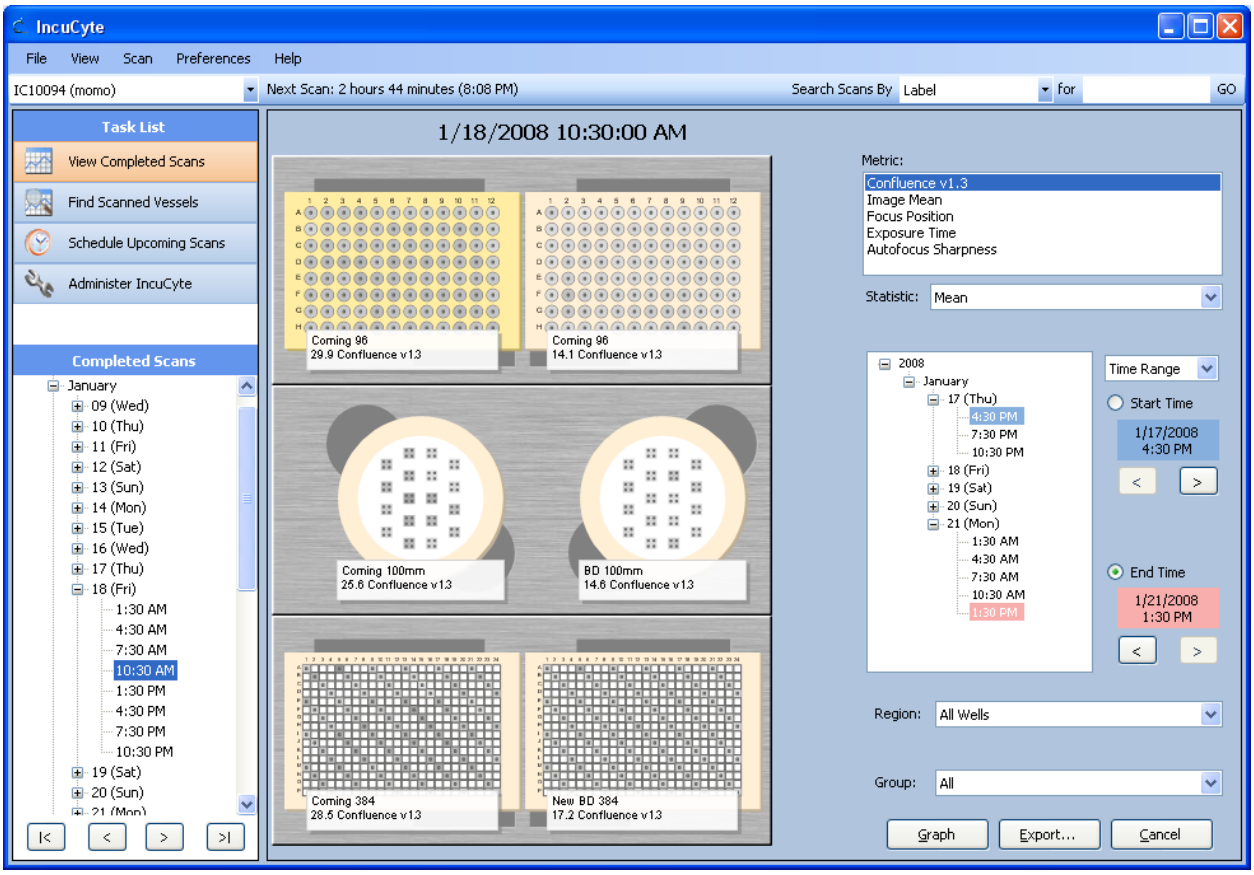


Figure 8-129. Ready to Graph

Press the Graph button. A graph will now appear in a new window (Figure 8-130). The graph will be assigned a default title, in this case All Mean vs. Time. The same graph can be obtained by using the Vessel View graphing option.

- Look at the graph in Figure 8-130. Because Region: All Sectors, Group: All was selected, only a single trace appears that represents the mean of all sectors in the dish. In Figure 8-131, a flask has been graphed. In this example, the Group: None option was checked, so one trace appears for each sector. When multiple wells/traces appear on a single graph, each one is listed at the top of the window (Figure 8-131). The wells/sectors will be assigned default titles indicating the vessel label and the well (A1, A2, A3, etc.).

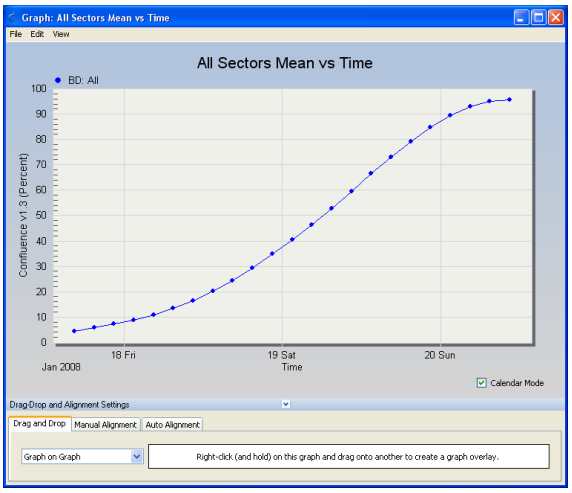


Figure 8-130. Dish Mean. Because Region: All Sectors, Group: All was selected, a single trace appears on the graph.

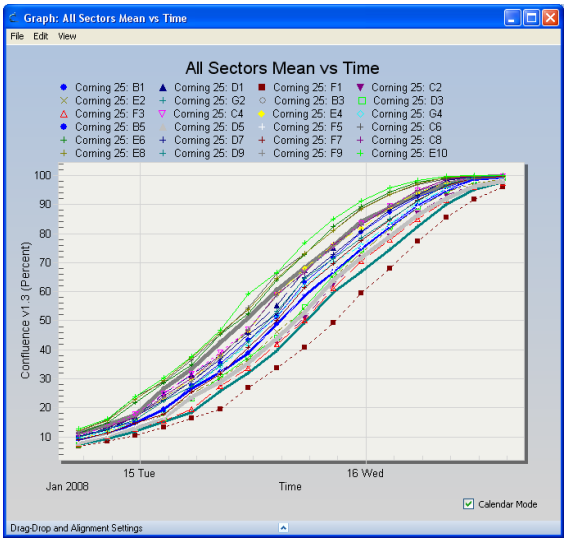


Figure 8-131. Flask All Sectors. The Group: None option was selected, so there is a separate trace for each sector. Each trace represents the image mean for that sector.

Graph Customization

Initial default labels along with other graph attributes can be personalized. Left click twice on the graph background area and the Customization Window will appear (Figure 8-132). This window can be used to set a variety of graph attributes including title, subtitle, axis, font, etc. Select the various tabs to explore all available graph-related features.

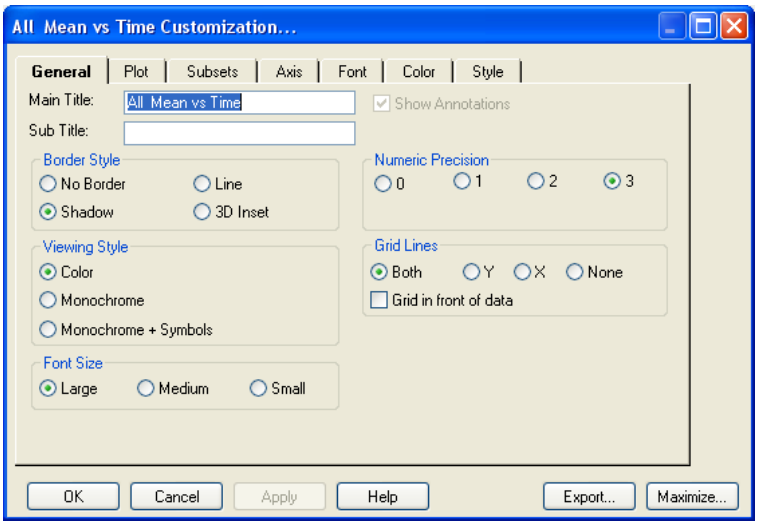


Figure 8-132. Graph Customization.

Individual well/sector names, however, CANNOT be changed using the Customization window. To assign new labels to wells or sectors, select the Edit option at the top left of the graph window. This will open the Titles and Labels window (Figure 8-133). This window provides an opportunity to change the Title and Subtitle, as well as the means to assign new well and sector labels. All default or previously set labels will appear in the Plot Labels box, while the currently highlighted label will appear below. To change a label, simply highlight the desired Plot Label with the mouse and then type the new label into the box underneath. Do not click OK until you are ready to close the Titles and Labels window. See Figure 8-133. Clicking on the Advanced button will bring up the Customization window (Figure 8-132). Additional customization options can be accessed by right-clicking on the graph background.

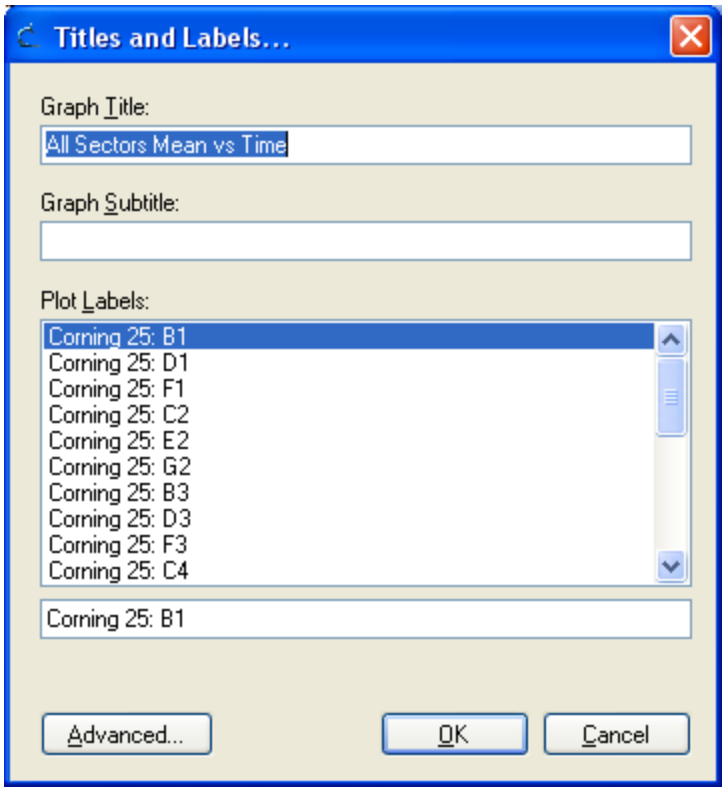


Figure 8-133. Titles and Labels Window

Drag and Drop Alignment Settings Task Pane

The Drag and Drop Alignment Settings can be visualized by opening the Tasks Pane at the bottom of the Graph Window. Click on the arrow on the blue bar at the bottom of the window to open or close the Tasks Pane. In Figure 8-130 the Tasks Pane is open and in Figure 8-131 the Tasks Pane is closed.

Dragging Graphs

There are two different circumstances under which graphs can be dragged using the IncuCyte™ software.

Dragging Graphs within the IncuCyte™ Software (Create Plot Overlays)

Under some circumstances, it will be convenient to combine traces created in separate graphs onto one single graph in order to facilitate a direct comparison. For example, the same cells were plated in two, separate flasks at the same starting confluence at the same time. How does their growth compare? Unfortunately, the mean growth curve of each flask must be generated separately, creating two, separate graphs. However, it would be optimal if both traces could be combined onto a single graph. How can this be accomplished? The two graphs in question are displayed in Figure 8-134 and Figure 8-135.

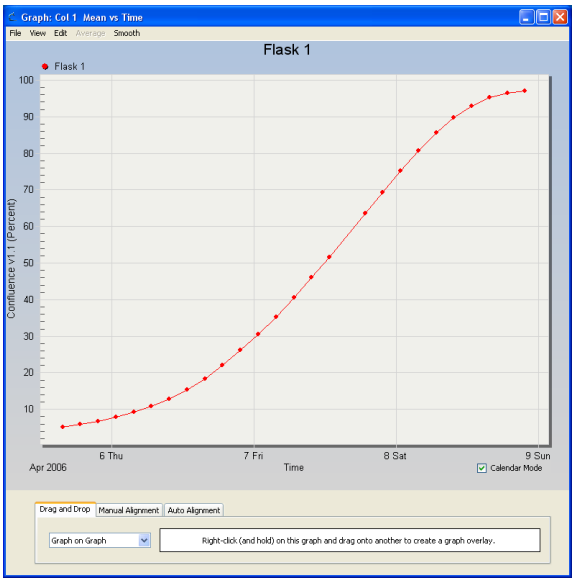


Figure 8-134. Graph for Flask 1

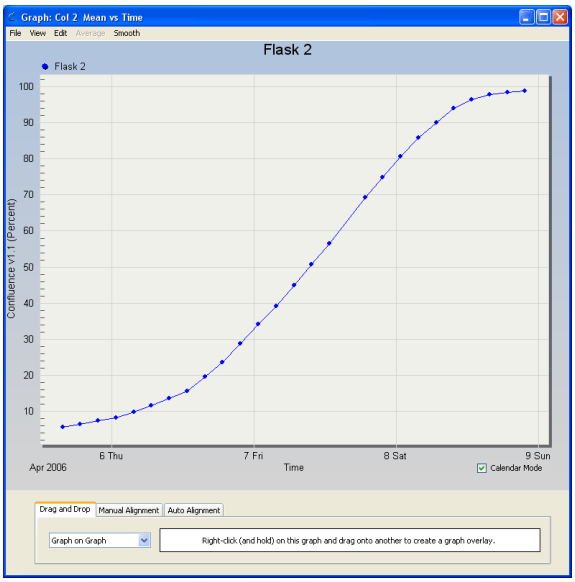


Figure 8-135. Graph for Flask 2.

As indicated in the Drag And Drop box at the bottom of the graph window, simply right-click (and hold) on the graph to be dragged, and then use the mouse to drag it onto the target graph. In this case, the graph for Flask 2 has been dragged onto the graph for Flask 1. The resulting graph is displayed in Figure 8-136. The new graph still contains the title from the original, Growth Curve for Flask 1; however, it now contains two traces labeled as Flask 1 and Flask 2.

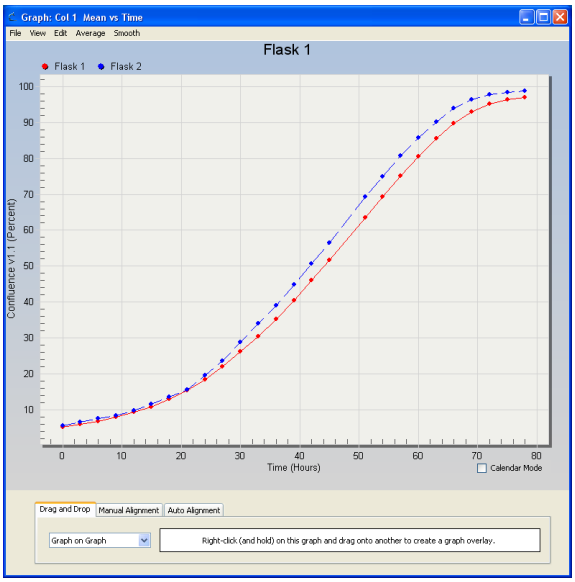

Graph: Col 1 Mean vs Time
File View Edit Average Smooth
Flask 1
Flask 1 Flask 2
Confluence v1.1 (Percent)
Time (Hours)
Calendar Mode
Drag and Drop
Manual Alignment
Auto Alignment
Graph on Graph
Right-click (and hold) on this graph and drag onto another to create a graph overlay.

Figure 8-136. Plot Overlay: Graph 1 + Graph 2

NOTE: When a graph Drag and Drop occurs, the entire graph is dragged. It is NOT possible to drag individual traces from within a single graph.

If only a single trace (for example, a single well from a 12-well plate) needs to be dragged, then it (the desired well) must be graphed alone and dragged individually. Alternatively, if the entire graph is dragged and dropped, individual traces can be deleted. See Section 8.10.5, page 192.

To drag and drop graphs onto each other within the IncuCyte™ software, be sure that the Graph on Graph selection is chosen from the Drag and Drop pull-down menu. If a graph overlay is attempted with the wrong Drag and Drop selection, a window will appear indicating that the overlay cannot occur.

There are 3 commonly-made errors that can prevent creating a graph overlay from occurring. If you are not able to drag your graph, be sure that:

- The correct Drag and Drop option is selected.
- The right mouse button is selected and not the left.
- You are not trying to drag the same graph onto itself.

Dragging Graphs from the IncuCyte™ Software into Different Software

It is also possible to drag a graph created within the IncuCyte™ software directly into a Word® document, EXCEL® file, PowerPoint® file or e-mail. In these cases, the graph can be dragged as either the raw data or the image. To drag the image into Word®, select Drag and Drop Image to Word or Outlook. To drag the graph image into EXCEL®, PowerPoint® or email, select Drag and Drop Image to Other Document. To drag the raw data from the graph, select Drag and Drop

Raw Data to Document. If the graph is dragged as Raw Data into EXCEL®, the data will appear neatly within the spreadsheet cells. See Figure 8-137. If the raw data are dragged into Word®, they will appear within columns.

Date Time	Elapsed	Column 1	Column 2
4/5/2006 15:45	0	5.116157	5.62277
4/5/2006 18:45	3	5.873641	6.483157
4/5/2006 21:45	6	6.748467	7.517222
4/6/2006 0:45	9	7.93768	8.310006
4/6/2006 3:45	12	9.286036	9.822143
4/6/2006 6:45	15	10.79348	11.6282
4/6/2006 9:45	18	12.91238	13.6381
4/6/2006 12:45	21	15.41242	15.66134
4/6/2006 15:45	24	18.40523	19.5973
4/6/2006 18:45	27	22.04792	23.65909
4/6/2006 21:45	30	26.11257	28.86244
4/7/2006 0:45	33	30.45502	34.0718
4/7/2006 3:45	36	35.27699	39.10634
4/7/2006 6:45	39	40.49081	44.93753
4/7/2006 9:45	42	46.00566	50.74551
4/7/2006 12:45	45	51.61444	56.49158
4/7/2006 18:45	51	63.54694	69.3348

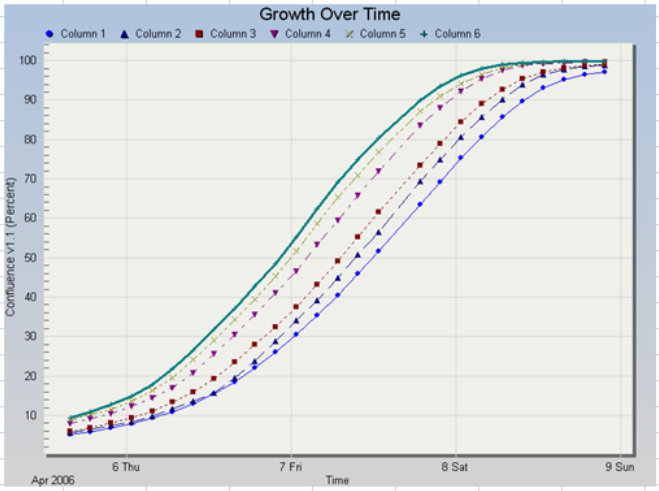


Figure 8-137. Graph data dragged into EXCEL® as Raw Data (left) and as Image (right).

The format in which the data will be dragged and dropped into the target software will be determined by the format in which the graph was created - for example, sorted by column vs. row.

SUMMARY:

1. To drag one plot onto another (within the IncuCyte™ graphing software) select Drag and Drop as Graph on Graph.

2. To drag the graph image into MS Word® or MS Outlook®, select Drag and Drop as Image to Word or Outlook.

3. To drag and drop the image into other software programs, select Drag and Drop as Image to Other Document.

4. To drag and drop other raw data, select Drag and Drop Raw Data to Document.

Preferences: Outlier Removal and Drag and Drop Auto Alignment

Select Preferences from the top of the main IncuCyte™ screen to open the Preferences/Outlier Removal Window. Two features of the Preferences window will be discussed here. See Figure 8-138.

- **Outlier Removal**: This function facilitates the increasingly stringent removal of outlying vessel data. Selecting Outlier Removal will reduce the standard deviation of the Metrics. The outlying data are selected automatically by the software according to an algorithm - points cannot

be excluded manually. If no outlier removal is desired, select None under the pull-down menu; however, standard is the default setting, and it is the setting recommended for general use.

NOTE: Increasing the stringency of Outlier Removal will not necessarily improve the appearance of a curve. To improve the appearance of a "noisy" curve, try using the Smooth Feature (page 197).

- **Graph Drag and Drop: Auto Alignment**. When graphs are dragged to create a plot overlay, they can be set to automatically align at a

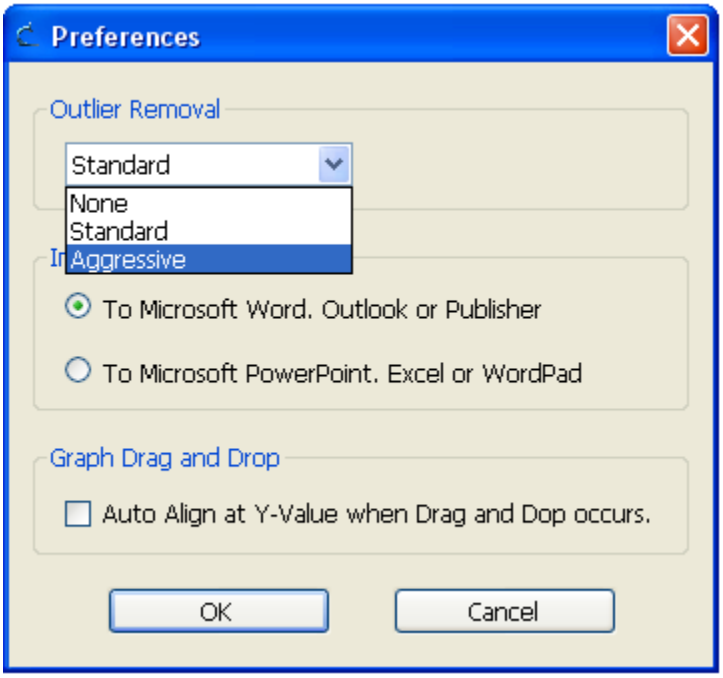


Figure 8-138. The Preferences Window

specific point on the Y-axis. Select the Drag and Drop: Auto Align at Y-Value when Drag and Drop occurs check box in the Preferences Window (Figure 8-138). Now, look at Figure 8-139. In the left graph, Graph Drag and Drop Auto Align was NOT checked. In the right graph, the Auto Align box WAS checked, and Auto Alignment was set to a Y-value of 22.

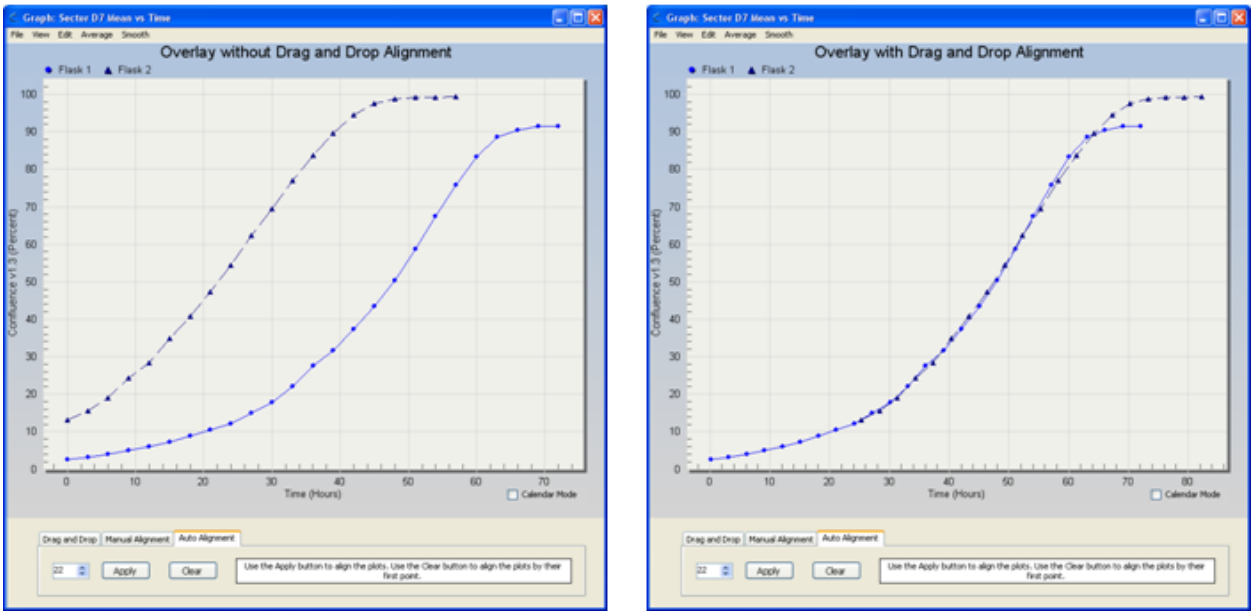

Overlay without Drag and Drop Alignment
Overlay with Drag and Drop Alignment
Flask 1
Flask 2
Time (Hours)
Drag and Drop
Manual Alignment
Auto Alignment
Apply
Clear
Use the Apply button to align the plots. Use the Clear button to align the plots by their first point.

Figure 8-139. Auto Alignment during Drag and Drop:

The Drag and Drop Alignment selection (Yes or No) chosen via the Preferences window will apply to all graph overlays, but the numerical value at which alignment occurs must be set using the graph window. See Figure 8-140. Select the Auto Alignment tab at the bottom of the graph window, and choose the value at which alignment will occur. The default value is 22, as displayed in Figure 8-140.

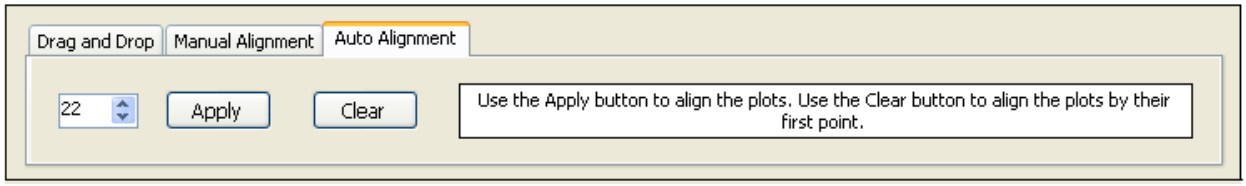

Drag and Drop
Manual Alignment
Auto Alignment
22
Apply
Clear
Use the Apply button to align the plots. Use the Clear button to align the plots by their first point.

Figure 8-140. Set the value at which Auto Alignment occurs.

Whatever alignment value is set under this tab will apply to all future graph overlays. To change the value, select a new value, and then press Apply. See Section Auto Alignment under Other Graph Features (page 189) for more information.

NOTES:

Outlier removal applies to ALL Metrics.

See Section 8.7.10 (page 156) for a discussion of the remaining Preferences Window feature.

Other Graph Features

Error Bars

When multiple wells or sectors are combined to generate a single trace, Error Bars can be added that represent the standard deviation between the wells or sectors. Error Bars can be added in one of two ways:

- Select View: Error Bars from the top of the main IncuCyte™ screen. When Error Bars are selected from the main screen, then all graphs generated subsequent to that time will automatically be assigned Error Bars. Deselecting the Error Bars will eliminate all Error Bars from subsequent graphs.
- Select View: Error Bars from the top of the graph window. When a graph window is open, the Error Bars option on the main screen will be inactive. As with the main View menu, all subsequent graphs will be affected by this selection.

Error Bars are ONLY available for multiple wells or sectors. They are NOT available for multiple images within a single well or sector

Two types of Error Bars are available:

Standard Error

Standard Deviation

Calendar Mode

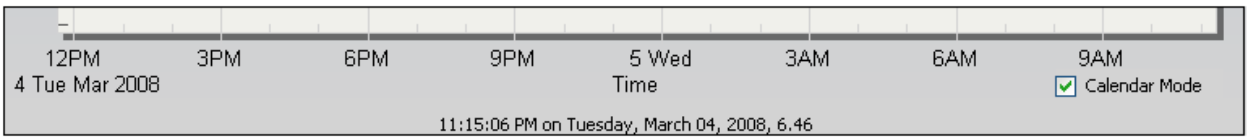


Figure 8-141. With Calendar Mode Selected.

At the bottom, right corner of the graph is the Calendar Mode check box. If the Calendar Mode box is selected, the x-axis of the graph will be displayed as year, month, day and time (Figure 8-141).

If Calendar Mode is NOT selected, the x-axis will be displayed in units of hours (Figure 8-142).

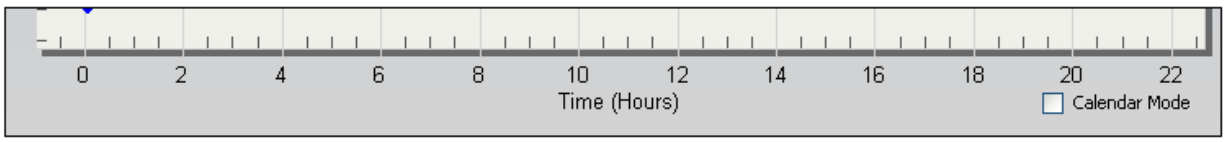


Figure 8-142. Calendar Mode NOT Selected.

When should Calendar Mode be used? If multiple graphs were created over the same time period, they can be dragged to create overlays in the Calendar Mode setting. This overlay facilitates a comparison of cell growth in absolute time. However, what if the same experiment was performed on different days? In this case, if both graphs are dragged together and then displayed in Calendar Mode, they will not overlap at all because they have no dates/times in common. To display these graphs properly overlapped, view the graph overlay with the Calendar Mode button NOT checked. Now, both graphs will be displayed as confluence relative to hours, irrespective of the specific days and times, and they will be overlapped (Figure 8-143 and Figure 8-144).

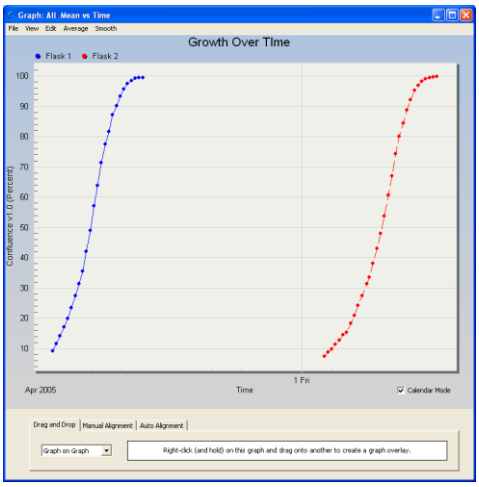


Figure 8-143. Two graphs from different days graphed onto each other with Calendar Mode checked.

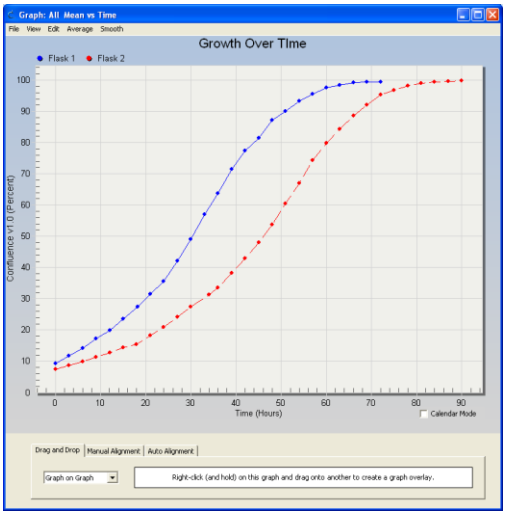


Figure 8-144. The same two graphs with Calendar Mode Unchecked.

Auto Alignment

NOTE: Also see Preferences: Outlier Removal and Drag and Drop Auto Alignment, page 184

At the bottom of the graph window, find the Auto Alignment tab. This tab can always be opened, but the Auto Alignment function is only active when the Calendar Mode button is NOT checked. In Figure 8-145, the auto alignment value is set to 14.

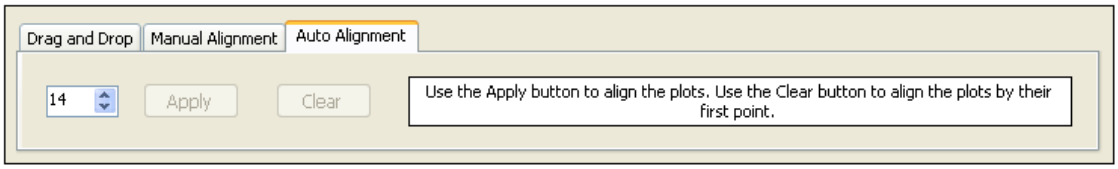

Drag and Drop
Manual Alignment
Auto Alignment
14
Apply
Clear
Use the Apply button to align the plots. Use the Clear button to align the plots by their first point.

Figure 8-145. The Auto Alignment Tab when the graph contains a single trace.

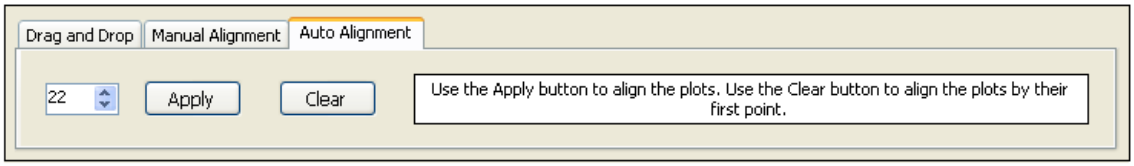

Drag and Drop
Manual Alignment
Auto Alignment
22
Apply
Clear
Use the Apply button to align the plots. Use the Clear button to align the plots by their first point.

Figure 8-146. The Auto Alignment Tab when the graph contains multiple traces.

When the IncuCyte™ software is first opened, this value will be set to the default number, 22. However, if the value is changed from this default, the new value will be displayed until it is changed again by the User, even if the program is closed and then opened again at a later date. If the Auto Align button is checked, then whenever graph overlays are generated by dragging, they will be aligned at this set value. When multiple traces are included on a single graph in Calendar Mode, their relative positions are constrained by the absolute times and dates of each point within the graph. However, when Calendar Mode is no longer checked, and the traces are displayed merely as confluence relative to hours of growth, individual traces can be aligned with each other according to a specific, selected percent confluence. Under what circumstances is such an alignment useful? Take for example, a situation where growth curves obtained from two separate flasks are to be compared. Unfortunately, however, the initial seating densities of the flasks were not the same. When the graphs are overlaid, the resulting graph appears as in Figure 8-147.

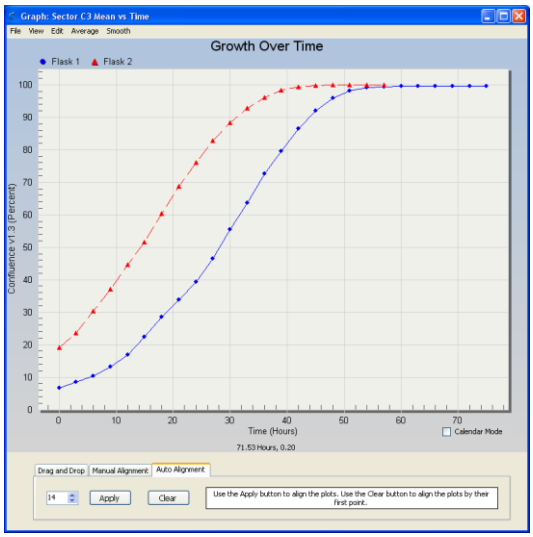


Figure 8-147. Plot Overlay with Auto Alignment set to 14.

In order to facilitate a more accurate comparison, reset the Auto Alignment value to one that is common to both graphs, for example, to 25. See Figure 8-148.

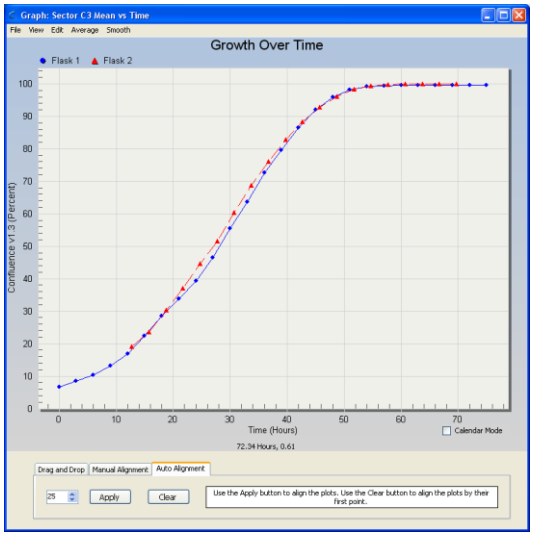


Figure 8-148. Resetting the Auto Alignment to 25

Now, the traces overlap appropriately, and growth can be more readily compared. Note, however, that the first point for both graphs is no longer set to 0 time as before. Because the graph for Flask 2 was dragged onto the graph for Flask 1, Flask 1 is the reference flask for this graph. Therefore, the hours-setting for Flask 1 remains at 0, but the hours-setting for Flask 2 is adjusted to compensate for the difference in starting confluence. So, Flask 2 growth now starts at ~ 12 hours.

If growth comparison is only relevant from the point of intersection on, then the early (low confluence) points for Flask 1 don't need to be displayed. Since it is clear at exactly which time point intersection occurs, the graph for Flask 1 can be re-graphed using the first intersection point as the first (start) time point. Repeat overlaying the graphs, and now the result displays both traces starting at 0 time at the point of intersection (see Figure 8-149).

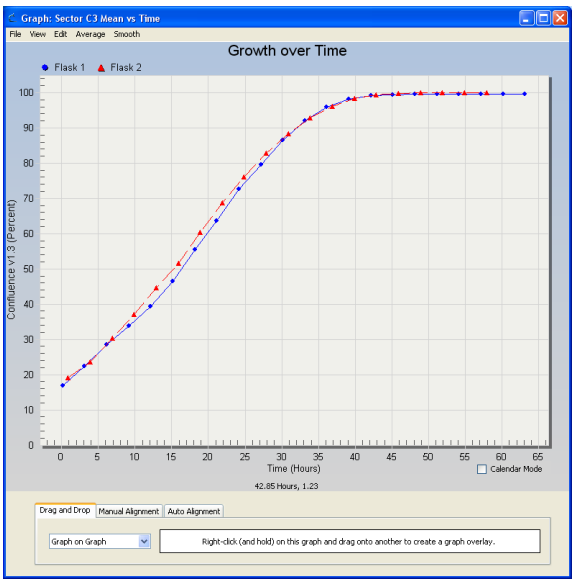


Figure 8-149. Both traces start at 0 time.

When the two disparate graphs were initially overlaid (see Figure 8-147) at Auto Alignment setting 14, the composite graph displayed both traces, one on top of the other. Even though an alignment value was set (14), no actual alignment was possible because the traces had no points in common (i.e. the trace for Flask 2 has no readings at 14% confluence).

IMPORTANT: When multiple traces need to be aligned onto a single graph, it is important to select an alignment point that is common to all of them.

If an alignment value is set that is outside the range of one or more traces, then the program cannot generate an accurate alignment. To set a new Auto Alignment, value, scroll through the values in the alignment box or simply type in a new value. Then press, Reset or hit Enter on the keyboard.

Another useful application of the Auto-Alignment function is the comparison of multiple traces at different stages of growth. In Figure 8-150 growth between two flasks plated under different circumstances is compared. When the traces are aligned at 15 (Figure 8-150) there is an obvious disparity in growth between the two samples. However, when the traces are aligned to 50, cells from both samples grow at a similar rate (Figure 8-151). Apparently, the cells in Flask 1

grow more rapidly at low confluence, but cells from both flasks grow at a similar rate once confluence reaches 50%.

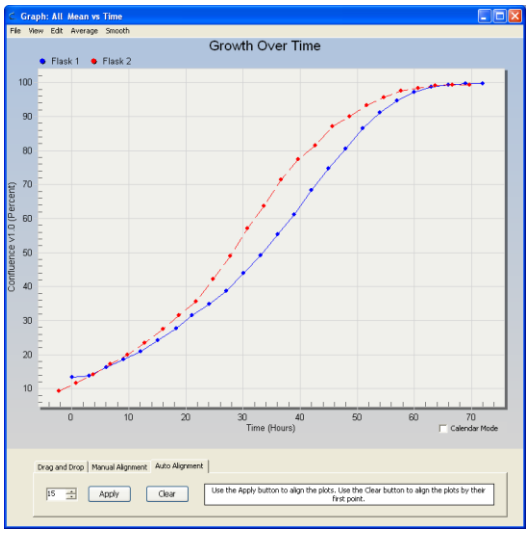


Figure 8-150. Alignment set at 15.

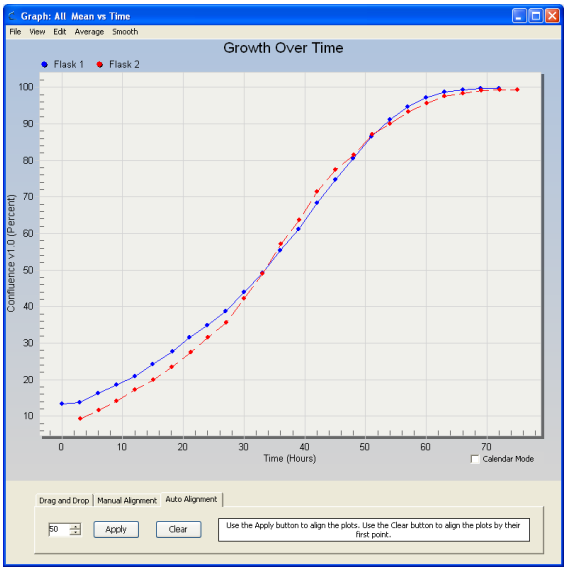


Figure 8-151. Alignment set at 50.

Manual Alignment/Deletion

This function enables manual alignment or deletion of each, individual trace on a graph. When the Manual Alignment tab is selected, the graph will initially be aligned at the value set in the Auto Alignment box. While the Auto Alignment function is used to align all traces on a graph to the same point, Manual

Alignment facilitates the alignment of individual traces upon a single graph at different points. In Figure 8-152, the graph consists of 3 traces, all aligned at 23% confluence. But the starting confluences differ between the traces. Align all three traces to the same starting confluence using the Manual Slider. Select each trace to be manipulated using the Trace Selection Box to the right of the slider. In Figure 8-152, Well 1 is selected. The selected trace can be pulled to the left and right using the slider. Use the slider to realign all three traces on the graph. See Figure 8-153.

NOTE: The advantage of the Manual Alignment slider over the Auto Alignment function is that Manual Alignment facilitates alignment of individual traces one at a time; whereas, Auto Alignment resets all traces simultaneously.

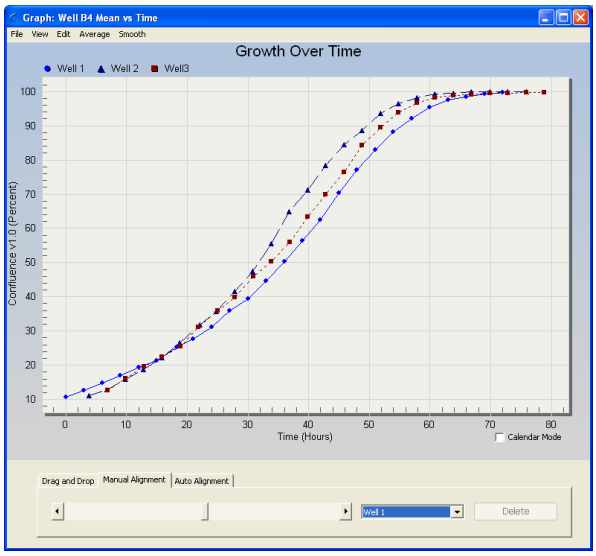


Figure 8-152. Manual Alignment set to 23. Note that Well 1 is selected in the Trace Selection Box to the right of the Manual Alignment slider).

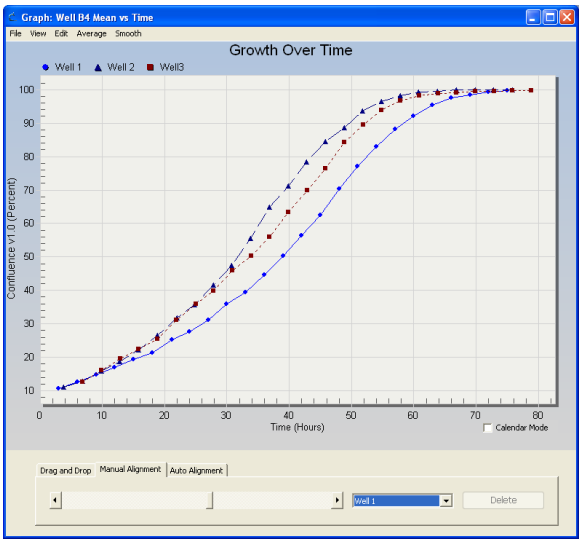


Figure 8-153. All three traces are aligned at the same starting concentration.

The Manual Alignment selection also facilitates deletion of selected traces. First, select the trace to be deleted by using the Trace Selection Box. Now, simply click on the Delete button. The Delete function can be used to delete a trace from any graph containing multiple traces - not just graphs created by dragging. For instance, all 12 wells of a 12-well plate have been graphed using the Graph All Wells selection. However, the trace from Well 6 needs to be eliminated from the graph. Select the Manual Alignment tab, use the pull down menu to select the trace for Well 6, and then click the Delete button. There are 2 circumstances under which traces from a graph CANNOT be deleted. These are:

- If the graph contains only a single trace.
- If all the wells or sectors from a vessel are graphed simultaneously, then the trace for the very first well or sector cannot be deleted. If all wells from a microplate are graphed simultaneously, this will be the trace for Well A1.

The above two deletion rules also hold true for the target graphs onto which plot overlays are created.

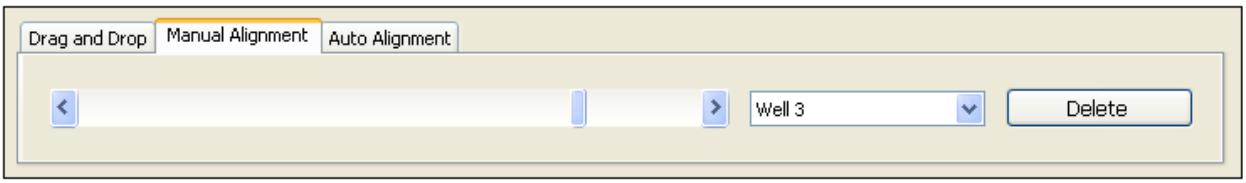


Figure 8-154. Trace Selection Box and Manual Alignment Slider

Selecting an Individual Point on the Trace

Use the mouse to select an individual point on a trace. Hold the mouse over the point, and the time and date of the scan represented by that point will be displayed. See Figure 8-155.

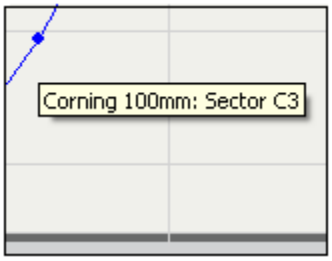


Figure 8-155. Hold the mouse over a specific point on the graph. The vessel label and the well/sector Connections will be displayed in a yellow box next to the point.

Double left-click on a selected point, and it will open the Vessel View window for that scan. The Vessel View window cannot be opened by double clicking on the points in an Average Graph.

The Graph Pull-Down Menu

Selecting the graph window pull-down menu presents 3 additional graph-related options.

File

Use the File Menu to open, save, or print a graph or to close the window (Figure 8-156. The File Pull-Down Menu).

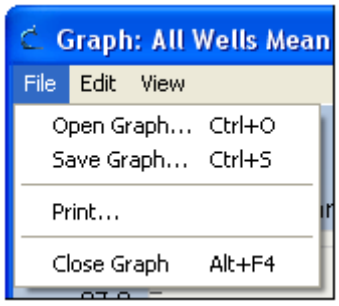


Figure 8-156. The File Pull-Down Menu

NOTE: If a graph was created in IncuCyte™, and then dragged and saved into another program (such as Word®), then the graph can be opened directly through that program. It will, however, be a static image that cannot be manipulated.

Edit

The Edit Menu provides the option to edit the graph title and labels associated with traces on the graph.

View

Use the View Menu to select different error measurements (standard error or standard deviation), change the y-axis to a log scale, or view filters from an analysis.

Tools

The Tools Menu provides options to edit how the data are viewed on the graph.

Smooth Lines: Sometimes, if the data are particularly noisy, the Smooth feature might be more useful than Outlier Removal. When Smooth is selected, a new window will appear to facilitate selection of the smoothing size, i.e., the number of points over which smoothing will occur. If 3 data points are selected, then each point on the graph will be averaged using its two neighboring points (Figure 8-157).

NOTE: It is important to remember that smoothing is a tool for generating cleaner curves when excessive noise prevents useful interpretation of the results. The smoothing feature should be used with discretion in order to prevent overt distortion of the data.

Estimate Rate of Change: When rate of change may assist data analysis (such as with cell migration assays, see Scratch Wound Assay Application Note), this option will provide an estimated rate for each point on the graph (197)

Average Lines Together: Create a graph showing the average of all plots on the current graph. This options can also be used to average plots from different vessels by first combining plots from different graphs using the Drag-Drop feature and then averaging the resulting graph.

NOTE: Error Bars are NOT available on graphs created using the Average function.

Each of these functions opens a new graph window, preserving the original.

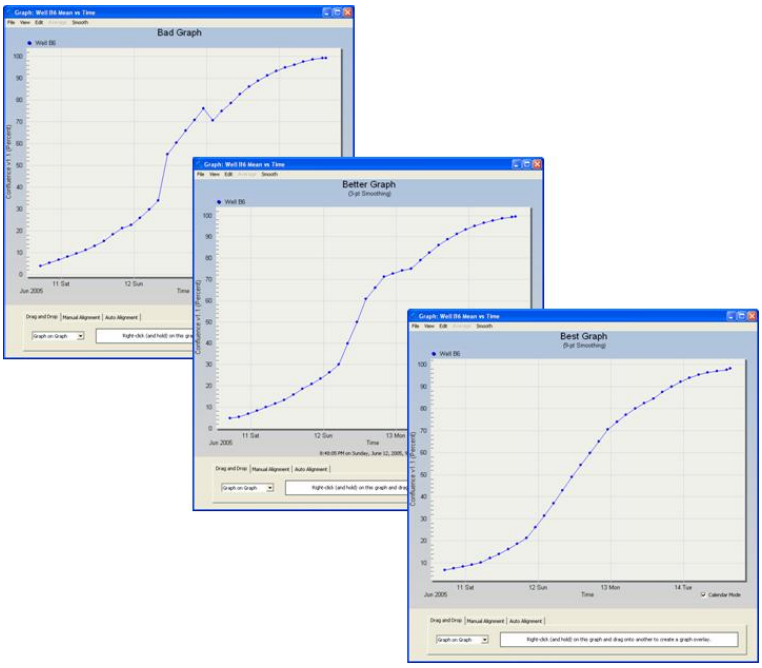


Figure 8-157. Results of graph Smoothing. The top, left-most graph is the original. The middle graph was smoothed with 3 data points selected. The bottom graph smoothed with 9 data points selected.

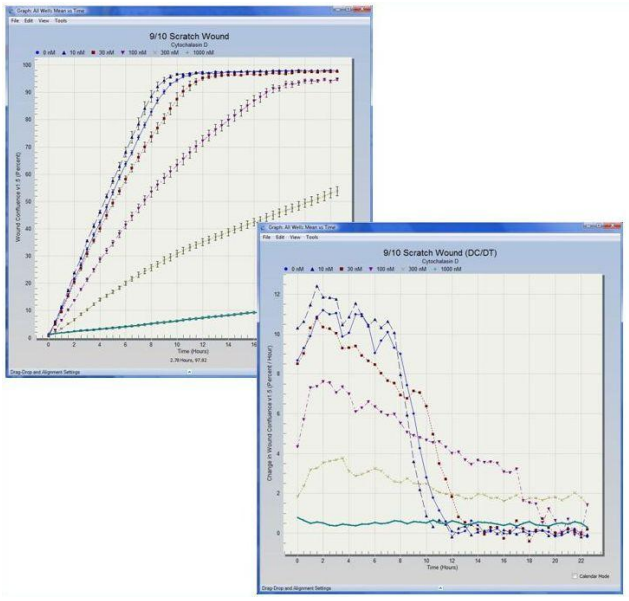


Figure 8-158. Results of the Rate of Change transformation. The top graph is the original – in this case a scratch wound experiment. The bottom graph shows the rate of chage at each data point.

Zooming the Graph

Zoom

Once a graph has been created, it is possible to Zoom into any particular area for closer inspection. To Zoom, left-click the mouse and then highlight the portion of the graph to be expanded. See Figure 8-159. Release the mouse to see the Zoomed portion of the graph. See Figure 8-160.

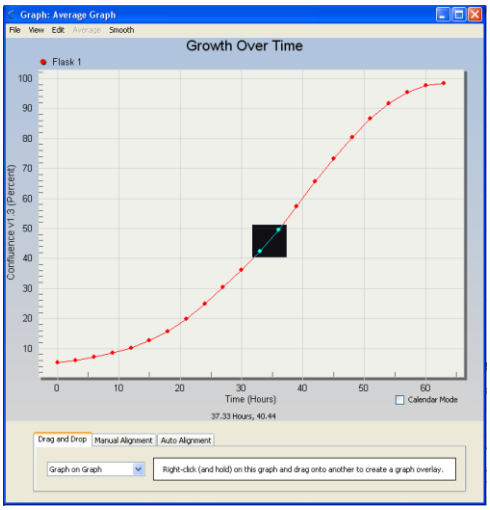


Figure 8-159. Highlight Area to Zoom

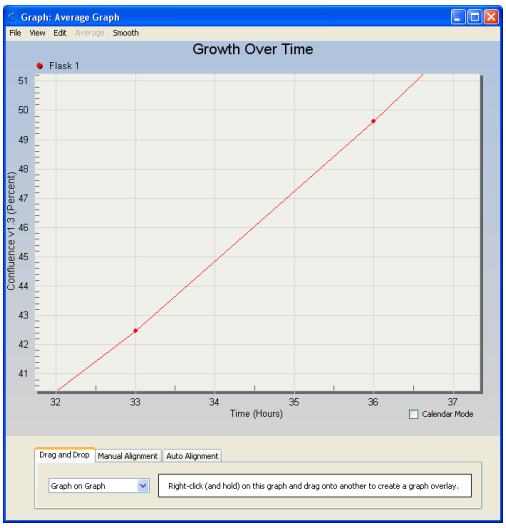


Figure 8-160. Zoomed Portion of the Graph

Undo Zoom

To reverse the zooming:

- Right-click on the expanded graph. A menu will appear. Select Undo Zoom from the menu, and the graph will return to its full-size format.
- Type the character “Z” on the keyboard.

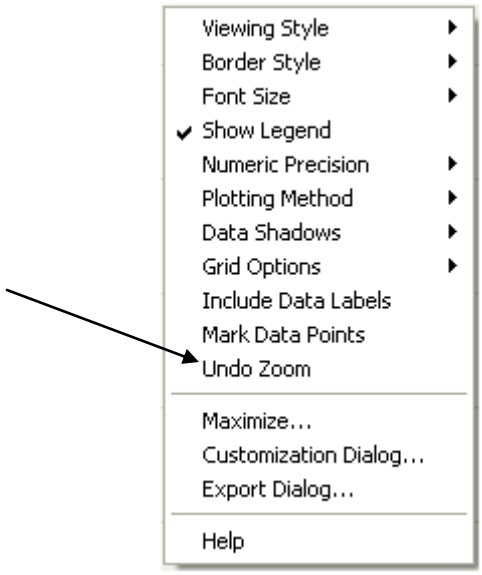


Figure 8-161. Undo Zoom

8.10.6 Microplate Graphing

The Microplate Graphing selection provides another option for graphing all experiments conducted in a microplate format.

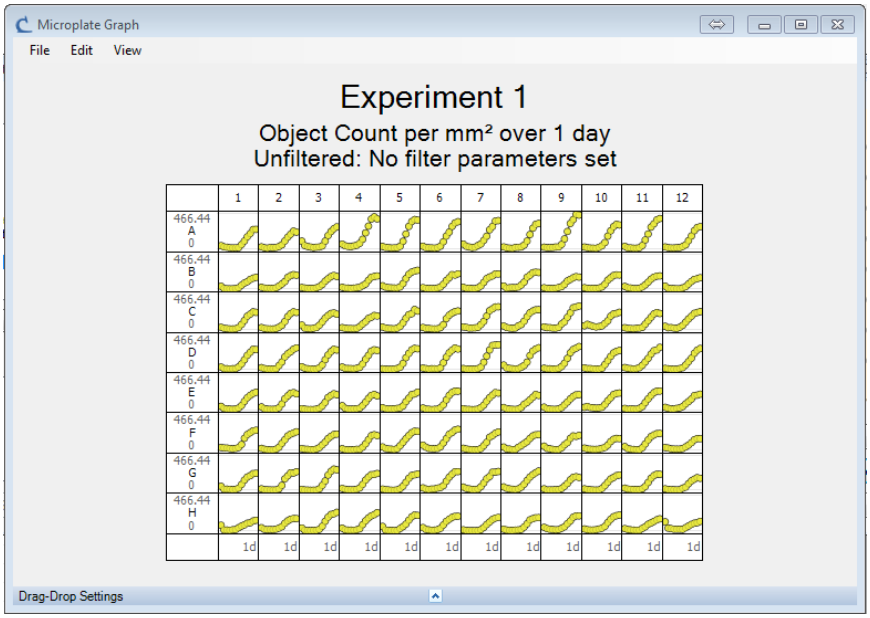


Figure 8-162. Microplate Graph.

Using the Microplate Graphing function, users can easily graph all wells individually in high or low density microplate. Unless selected otherwise, the Microplate Graphing color scheme directly mirrors the information in the Plate Map editor. Furthermore, Microplate graphing can be customized using the Menu options. These options include:

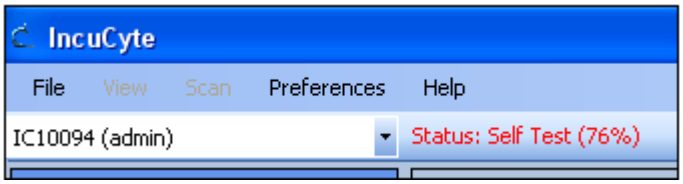
- Editing Titles/Subtitles
- Axis Range Control
- Show/Hide x and y axis labels
- Logarithmic y-axis scaling
- S/M/L marker sizes

In addition, users can export images of the Microplate Graph by selecting one of 4 different file types (PNG, TIFF, JPEG, or BMP) and selecting one of 5 different predetermined file sizes. Print and Print Preview and Drag and Drop functionality have also been added to the Microplate Graphing option.

8.11 The IncuCyte™ Self-Test

The IncuCyte™ will perform a device Self-Test every 5 hours if it is not in regular use. Therefore, if insufficient scanning is taking place, or if scans are scheduled at an interval of greater than 5 hours, Self Tests will occur. If a Self-Test is ongoing, the status of the test will be displayed in red at the top left of the main window, and the green Scan Active light on the front panel of the IncuCyte™ panel will be lit. It is important to note that the Self-Test is completely normal behavior, and it does NOT indicate that there is any problem with IncuCyte™ function. While the Self-Test cannot be disabled, it can be terminated by either:

- Starting a scan during the Self-Test or
- Pressing the red Stop button on the front panel of the IncuCyte™



8-163. Device Self Test

FLR

9 IncuCyte™ FLR

The IncuCyte™ FLR is an HD-enabled instrument that is also equipped with fluorescence scanning capabilities. When vessels are scanned in Fluorescence & Phase-Contrast mode, both an HD and fluorescence image will be generated. Alternatively, the IncuCyte™ FLR can also scan images in HD mode alone (i.e. non-fluorescently).

9.1 The IncuCyte™ FLR Vessel View Window

Fluorescent images can ONLY be viewed and manipulated using the Vessel View window. See Figure 9-1. Therefore, this window plays a more important role in fluorescence imaging relative to non-fluorescence imaging.

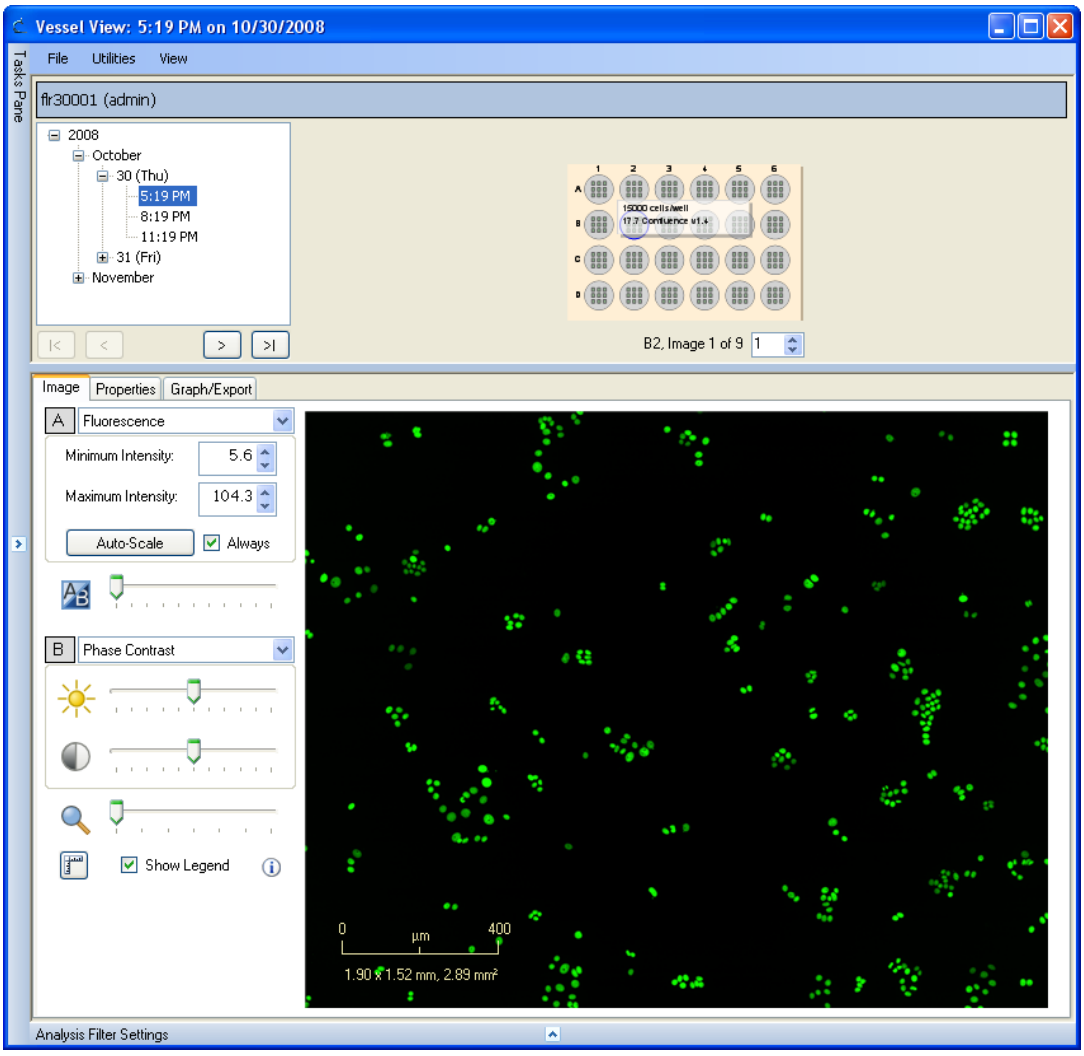


Figure 9-1. The Fluorescence Vessel View Window

9.2 Viewing Fluorescent Images in the IncuCyte™ FLR

Fluorescence scanning generates both an HD (phase contrast) and fluorescence image at each scan pattern location. Open the Vessel View window and select the Image tab. The Image tab in the FLR Vessel View Window provides the User with a selection of viewing options. It is divided into two Channels: A and B. Use the corresponding pull-down menus to make your selections. See Figure 9-1. You can select either Phase Contrast or Fluorescence for each channel. In Figure 9-1, Channel A is set to Fluorescence, and Channel B is set to Phase Contrast. The two channels are independent, and the settings for each channel can be independently adjusted. However, the images from the two channels can be overlayed using the A/B Slider Bar to form a single, composite image. See The A/B Slider, page 204.

9.2.1 Fluorescence Settings

The Fluorescence settings allow the User to adjust the DISPLAY characteristics of fluorescence components within an image. Adjusting the display settings will affect which components of an image will be displayed as fluorescent, and also the brightness of those fluorescent images relative to each other. It will NOT affect the absolute (calibrated) fluorescence values. Calibrated fluorescence values are measured in terms of pixel brightness using Arbitrary Units (AU's).

Figure 9-2 shows the case of Channel A and B both set to Fluorescence. The "mouse over" Pixel Intensity for fluorescence image NEVER CHANGES. The Pixel report will change based upon whether or not an image has been processed.

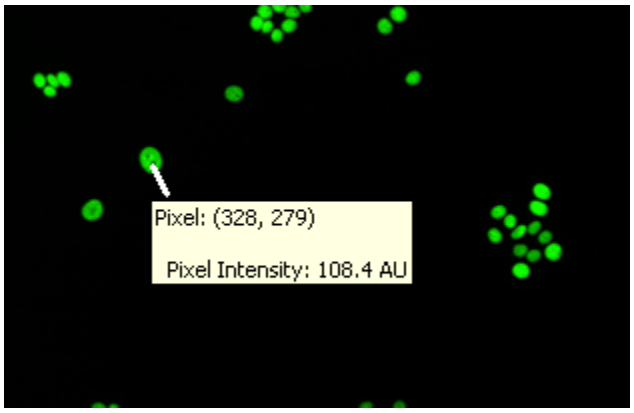


Figure 9-2. View Pixel Intensity and Coordinates

Selecting a Minimum and Maximum Intensity basically "clips" the brightness at which fluorescent components will be displayed. In Figure 9-1, the Maximum Intensity is set to 104.3. Nonetheless, it is possible that the image will still contain areas with calibrated pixel values greater than 104.3. However, these areas will be assigned the SAME degree of brightness in the display as an area with a pixel value equal to 104.3. Thus all areas with a Pixel Intensity of 104.3 or greater will be displayed as equally bright. To increase the dynamic range of brightness displayed for areas at 104.3 AU and above, increase the value of the Maximum Intensity.

Minimum Intensity:

Any portion of the image with a pixel intensity value less than or equal to the Minimum Intensity will be assigned a relative brightness equal to the darkest possible value in the image (in this case black).

Maximum Intensity:

Any portion of the image with a pixel intensity greater than or equal to the Maximum Intensity will be assigned a relative brightness equal to the brightest possible value for the display (in this case, bright green).

All portions of the image with pixel intensities between the Minimum and Maximum Intensity values will be assigned intermediate degrees of brightness.

The fluorescence Min/Max Intensity settings can be adjusted using the Auto-Scale function or via Manual Adjustment.

Setting Fluorescence Intensity Values using the Auto-Scale Button

Autoscaling tries to assign optimum Min/Max settings for a particular image. Autoscaling is easy (just press the Auto-Scale button), and in many cases the Auto-Scale settings will be adequate.

Setting Fluorescence Intensity Values using Manual Adjustment

Either type new values directly into the Min/Max intensity boxes or and press the Enter key, or use the arrows to scroll through values. Use the mouse to view pixel intensities of the background and of fluorescent areas to determine the settings that best accommodate your image.

Whether Min/Max settings are set manually or via the Autoscale function, they will remain unchanged until manually readjusted or re-autoscaled on a different image. Therefore, the Min/Max settings applied to the original image will be applied to all subsequent images that are viewed within the same vessel at all scan times or even to images from different vessels. If images are combined to generate movies, each frame of the movie will contain an image with the same Min/Max settings.

Always Autoscale

There are times when the autoscaled Min/Max settings for one image will no longer represent the best settings for other images. If the Always box next to the Auto-Scale button is checked, then every time a new image is selected it will be automatically Auto-Scaled rather than using the values from the previous image. If a movie is generated, each image within the movie can be individually autoscaled. In Figure 9-1, the Auto-Scale Always box has been selected. Note the Always button ONLY applies to Auto-Scaled values. For more information regarding generation of autoscaled movies, see Section 8.7.7, Export Images and Movies FLR, page 150.

9.2.2 Phase Contrast Settings

Phase contrast image Brightness and Intensity can be adjusted using the appropriate slider bars.

9.2.3 The A/B Slider

Dragging the A/B Slider generates a single image composite overlay of the two images from Channels A and B. The User selects a Channel A image and then a Channel B image, and then uses the A/B slider to select the blending ratio. When the slider is positioned at the far left, the image displayed is 100% of Channel A and 0% of Channel B. Analogously, when the slider is at the far right, 100% of Channel B is displayed. A 50/50 blend is achieved when the slider is centered.

In addition to blending Fluorescence and Phase images together, the Fluorescence Threshold Blending feature defines an intensity threshold for combining the Fluorescence image with the Phase Contrast image; pixels above the threshold are taken from the Fluorescence image, while pixels below the threshold are taken from the Phase Contrast image. In this way, fluorescent areas of an image show up brighter than when simply blended with the phase image, while the Phase Contrast portion of the image retains its original brightness and contrast settings.

Initiate Fluorescence Threshold Blending either by left-clicking on the A/B slider icon or by selecting it in the View menu at the top of the Vessel View window. (Notice the A/B slider now reads “Threshold” instead of “Blend” under the A/B icon.) With the slider all the way toward the Fluorescence channel, the Fluorescence image appears with the current Max/Min settings. Dragging the slider toward the Phase Contrast channel will allow only those brighter areas of the Fluorescence image to appear overlain on the Phase Contrast image. With the slider all the way toward the Phase Contrast channel, any areas of the Fluorescence image brighter than the Maximum Intensity setting will remain in the image.

9.3 IncuCyte™ FLR - Specific Software Applications

There are some image analysis applications that are unique to the IncuCyte™ FLR, and they can only be accessed through the FLR Vessel View window. These applications are:

Tech Note

The Object Counting Application

The Angiogenesis Application

The Object Counting Application is a standard component of the IncuCyte™ FLR software. However, the Angiogenesis Application is an independent software module that must be purchased separately. An overview of both applications will be provided in this manual.

The circumstances under which these two applications would be applied are very different; however, both of them operate from the same basic platform. In this regard, they share many common elements. An overview of these common elements will be provided first, followed by some basic definitions specific to each application. For more in-depth information please refer to the corresponding application notes or contact Essen BioScience for assistance.

Some important definitions are included below:

Analysis Job

Quantitative image analysis using an FLR software image analysis application.

Segmentation

A fundamental basis for FLR image analysis is the separation of fluorescent (foreground) and non-fluorescent (background) objects. This process is called Segmentation. The User can adjust and optimize the parameters utilized to assign Segmentation to an image.

It is important to choose Segmentation Parameters that most accurately represent those components of the image that will be subject to analysis.

Segmentation Mask

Segmentation can be visualized in the form of a Segmentation Mask. Areas of an image that have been identified as belonging to the foreground will be indicated with a white mask. Areas that have been identified as belonging to the background will be black.

In Figure 9-3, a Segmentation Mask (right) has been generated from a raw, fluorescent image (left). The Segmentation parameters have been adjusted such that the mask accurately represents the fluorescent components of the image.

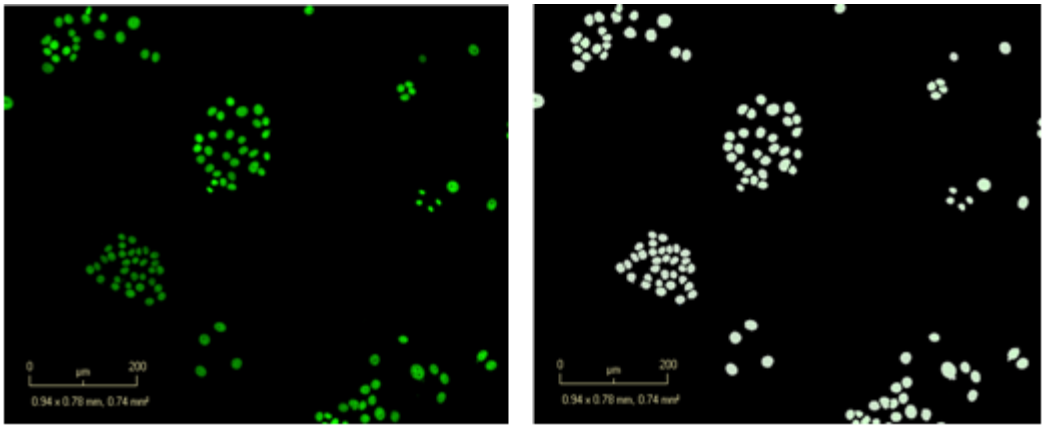


Figure 9-3. Application of Segmentation Mask. The raw image is on the left and the corresponding Segmentation Mask is on the right.

9.3.1 The IncuCyte™ FLR Tasks Pane

Open the Vessel View window and then open the Tasks Pane by clicking on the left edge of the window. See Figure 9-4. Use the Tasks Pane to preview and run Analysis Jobs.

9.3.2 Task Bars Available in the IncuCyte™ FLR Tasks Pane

Four Task Bars are available in the FLR Tasks Pane.

Utilities

The Utilities Task Bar in the IncuCyte™ FLR Vessel View Tasks Pane is the same as in the non-fluorescent IncuCyte™. See Tasks Pane, page 133.

Analysis Jobs: Open and Manage

All Analysis Jobs that have been run, or are currently running, will be displayed in this window. Opening and Managing Analysis Jobs will be discussed further in Analysis Jobs: Open and Manage, page 206.

Object Counting: New Analysis

Optimize (preview) the parameters for Object Counting and launch a new analysis. See Section 9.3.5, page 215. The opened Object Counting Task Bar is displayed in Figure 9-4.

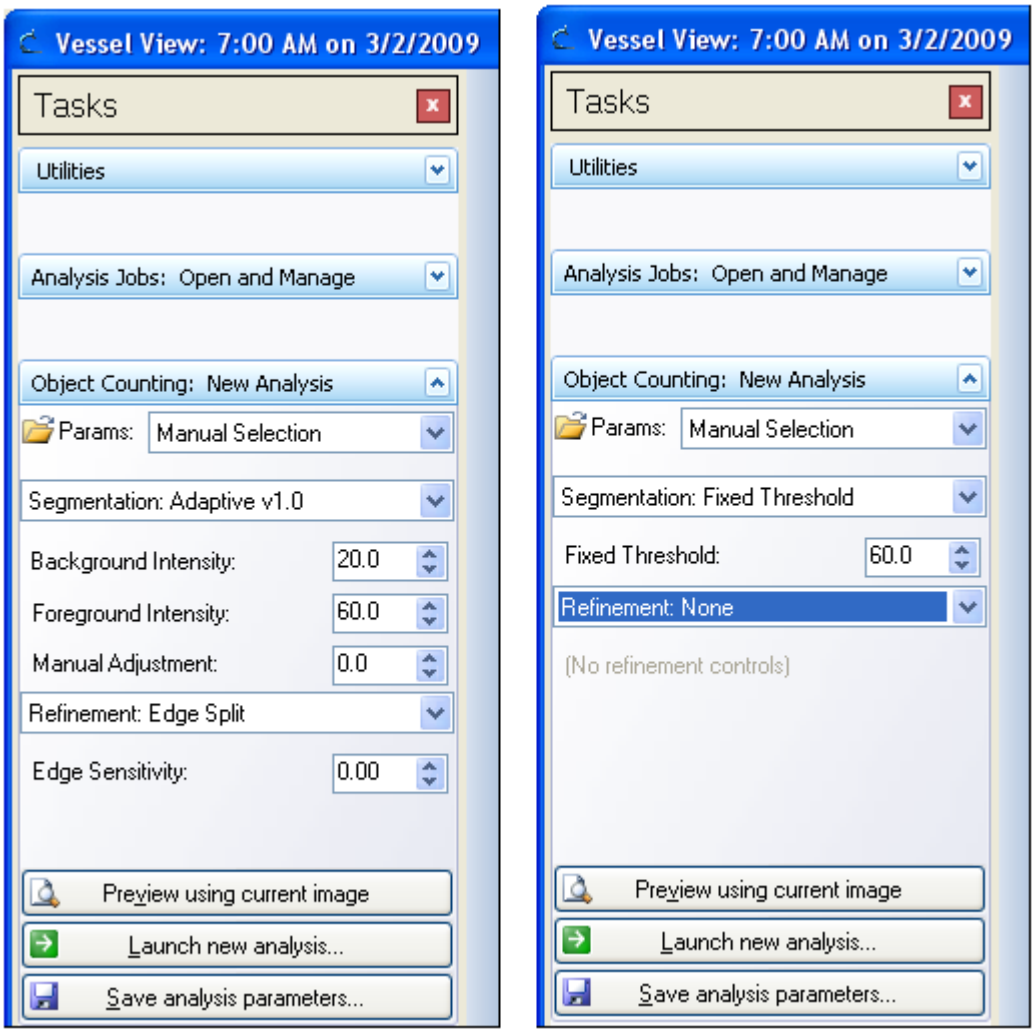


Figure 9-4. The IncuCyte™ FLR Tasks Pane

Three Task Bars are visible: Utitilies, Analysis Jobs: Open and Manage and Object Counting: New Analysis. Only the Object Counting Task Bar is open.

In the left image, Adaptive Segmentation and Edge Split Refinement have been selected. In the right image, Fixed Threshold Segmentation and no Refinement have been selected.

Angiogenesis: New Analysis

Optimize (preview) the parameters for Angiogenesis Analysis and launch a new analysis. See Section **Error! Reference source not found.**, **Error! Reference source not found.**, page **Error! Bookmark not defined.**.

NOTE: The Angiogenesis Task Bar will ONLY be available if the User has purchased the Angiogenesis Module.

9.3.3 Analysis Tools Available in the IncuCyte™ FLR Tasks Pane

All analyses must be launched from the Vessel View window Tasks Pane. There are typically two steps to running an Analysis Job:

Use the Preview Current Image button to optimize Analysis Parameters. Additional information and analysis strategies are provided in a Technical Note that can be downloaded from the Essen BioScience web page.

Launch a new Job Analysis.

Open the Object Counting Task Bar. See Figure 9-4. Opening this Task Bar allows the User to adjust image Analysis Parameters, to preview and launch Analysis Jobs, and to save Analysis Parameters.

There are three pull-down menus available related to setting Analysis Parameters:

Parameters: Abbreviated in the Tasks pane as Params

Segmentation

Refinement

There are three buttons available related to previewing, launching jobs and saving Parameters:

Preview using Current Image

Launch New Analysis

Save Analysis Parameters

The Parameters Pull-Down Menu

If no Analysis Parameters have been saved, then the only option under the Parameters pull-down menu will be Manual. However, it is possible to select and then save Analysis Parameters. Saving Analysis Parameters will increase the options available under the Parameters pull-down window.

Any saved Analysis Parameters will now be available. They can be selected and opened.

A new option will appear in the pull-down window called Manage Analysis Parameters. Select this option to delete or rename saved Analysis Parameters.

When Manual is selected, the User is free to adjust the Segmentation and Refinement settings. If saved Parameters have been selected, then the Segmentation and Refinement values will be populated accordingly. However, adjusting any of the Segmentation and/or Refinement Parameters will cause the saved Parameter selection to jump back to Manual.

The Segmentation Pull-Down Menu

Segmentation refers to the separation of foreground and background components of an image, and it leads to the identification of Objects within the image area. Objects belong to the foreground.

Object: An isolated region within the image that stands out from the background.

Two options are available with regard to Segmentation:

Fixed Threshold

Adaptive Threshold

Segmentation: Fixed Threshold

Choosing Fixed Threshold applies a single, User-selected threshold value for all images included in the analysis. Pixels with intensities less than this value will be classified as background. Pixels with intensities greater than or equal to this value will be classified as foreground.

Segmentation: Adaptive

Adaptive Segmentation uses an algorithm to determine a good threshold for each image, and it also uses different thresholds within an image. This option allows the software to compensate for variations in the background fluorescence both within and between images.

If Adaptive Segmentation is selected the User must choose values for:

Background Intensity:

Value of the fluorescent image outside of an object. All areas within a fluorescently scanned image are assigned a fluorescence value (represented as pixel intensity). The User must decide which fluorescence values should be classified as background.

Foreground Intensity:

Value of the fluorescent image inside an object.

Manual Adjustment:

Value added to the threshold determined by the adaptive algorithm before the threshold is applied to the image. Positive Manual Adjustment will help to remove background (both areas adjacent to Objects and areas distant from Objects). Negative Manual Adjustment will assist with the identification of faintly fluorescent Objects.

The Background and Foreground Intensities determine the range over which the software searches for a threshold, and they do not need to be very precise.

Tech Note

For recommendations on how to reduce background fluorescence, see the Essen BioScience application note on this topic.

The Refinement Pull-Down Menu

The Refinement option allows the User to adjust the degree of Refinement (or separation) between adjacent Objects.

Refinement: Edge Split

Edge Split values have a range of -1.00 to 1.00. Based on the Edge Split value, the software makes an attempt to split (or separate) Objects that are close together. Positive values will cause more Objects to be split. Alternatively, negative values will result in fewer Objects being split.

Refinement: None

No attempt will be made to separate adjacent Objects.

Preview Using Current Image Button

Pressing the Preview button allows the User to run an analysis on a single, currently selected image in order to preview/evaluate the Analysis Parameters. The Preview function will be more fully discussed in Section 9.3.4, page 211.

Launch Analysis Button

Pressing this button will launch an Analysis Job for all selected images based upon the selected Analysis Parameters. See Section 9.3.5, page 215 for more information.

Save Analysis Parameters Button

The User may define Analysis Parameters that will be applicable to multiple experiments. Rather than having to manually reset these same parameters each time an analysis is launched, parameter settings can be saved, named and reopened at a later time. Use the Save Analysis Parameters button to save all parameter values that are currently selected. A new window will appear to give this set of parameters a name. Saved Parameters will be available under the Parameters pull-down menu. Additionally, Saved Parameters can be renamed or deleted by selecting Manage Analysis Parameters from the Parameters pull-down menu.

Vessel View FLR Banner

The colored banner at the top of the Vessel View window is color-coded to reflect changes in the current viewing mode. See Figure 9-5.

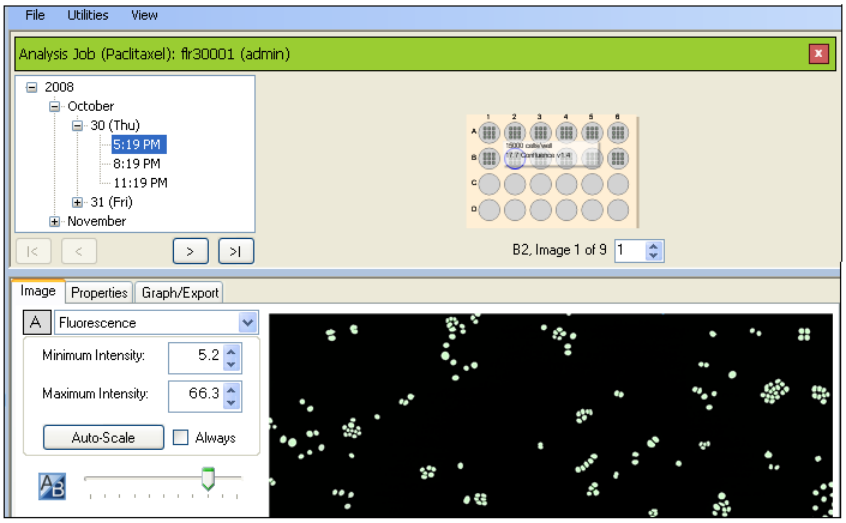


Figure 9-5. Open Analysis Job has Green Banner

Changes in the banner color are intended to alert the User to which mode is currently selected. These modes will be explained in more detail in the following sections; however, a summary of the banner colors is as follows:

Standard Mode: Blue

Job Preview Mode: Beige

Unapplied Preview Mode: Yellow

Complete Analysis Job: Green

In Figure 9-5 the banner is green indicating that a complete Analysis Job has been opened. The name of the Analysis Job is Paclitaxel.

9.3.4 Preview Using Current Image (Analysis Preview Mode)

Use this button to launch an analysis of a single, currently selected image. Depending on the size of the data set, running a complete Analysis Job can require a significant amount of time. Additionally, once an Analysis Job has been completed, it is NOT possible to adjust any of the Segmentation or Refinement Settings. Therefore, it is best to use the Preview function to optimize all Segmentation and Refinement Parameters prior to running an analysis of a full-length experiment.

IMPORTANT: It is NOT possible to adjust the Segmentation or Refinement Parameters of a completed Analysis Job.

To launch a preview, select a representative image and then press the Preview Current Image button. See Figure 9-6, page 214. Pressing the Preview button puts you in Analysis Preview Mode. Note that several things will happen immediately upon pressing the Preview button.

The Preview Current Image button is renamed to Update Analysis Preview

The Banner at the top of the Vessel View window changes from blue to beige, and it reads: Analysis Preview

The Analysis Filter Settings Pane at the bottom of the Vessel View Window is activated.

Object Segmentation Mask becomes one of the options in the A and B Channel pull-down menus

Holding the mouse over Objects within the image has the potential to display additional information that was not available in unprocessed images. See Object Properties: Definitions, page 224.

There are 4 ways to exit Preview mode:

Navigate to a different image or to the same image at a different scan time. Even though all scans associated with that vessel will be displayed in the Time Tree, the preview still only applies to the currently selected image.

Click on the red “X” at far right end of the yellow banner

Open an Analysis Job

Change a Parameter Setting

Changing a Parameter Setting will launch Unapplied Preview Mode. See Unapplied Analysis Preview Mode, page 215.

Graphing and Export in Analysis Preview Mode

In Preview Mode, single-time export of the selected image is available, and in some cases histograms can be generated.

Navigate to the Graph/Export tab and view the selections in the Metric pull-down menu. The Metric pull-down menu will now include new options associated with processed data (for example, Object Count per Well). The data corresponding to the Metric for this single image will be displayed in the Graph/Export tab.

Select the Graph/Export button to open the Graph/Export Window. The Time-Tree in this window will include a single date for the one image that was previewed. Because only one image from one time point can be previewed, it is NOT possible to create a time plot in Preview Mode. However, it is possible to export the data associated with this single image, and in some cases it is possible to generate a histogram. See Graph/Export Histograms of Processed Jobs, page 232 for information on graphing histograms.

Note that the Graph/Export window includes a list of any filters that have been applied to the image. Use the Include Filter Details checkbox to apply the Filter Settings to any graphed or exported data. Uncheck the box to graph/export the data with no filter settings applied. See Section 9.3.8, Filter Settings, page 224 for information on Filter Settings.

It is important to remember that in Analysis Preview Mode, all analysis settings are applied ONLY to the currently selected image. Selecting a different image, or the same image at a different scan time, will cause you to exit Preview Mode.

In Figure 9-6, the Object Segmentation Mask is being applied to a cell line which expresses GPF in the nucleus, so each Object represents a nucleus. If the goal is to launch an analysis that will count all the nuclei in the image, then the Segmentation and Refinement Parameters should be adjusted such that the Segmentation Mask identifies the fluorescent nuclei visible in the fluorescence image as accurately as possible, both in terms of size and number. The degree to which the two images overlap can be evaluated using the A/B Slider Bar. In Figure 9-6, Channel A is set to Fluorescence, and Channel B is set to Object Segmentation Mask. Because the Slider Bar is pulled all the way to the right, the image displays 100% Segmentation Mask. However, if the Slider Bar is pulled to the left, the image blend will shift to include more of the Fluorescence Image. Use this image overlay function to evaluate how accurately the Segmentation Mask is identifying the fluorescent nuclei in the image. For a closer inspection, use the Zoom slider.

To determine the best Analysis Parameters that will mostly accurately reflect all images within the experiment, it is best to preview multiple images that represent different experimental conditions and/or different time points.

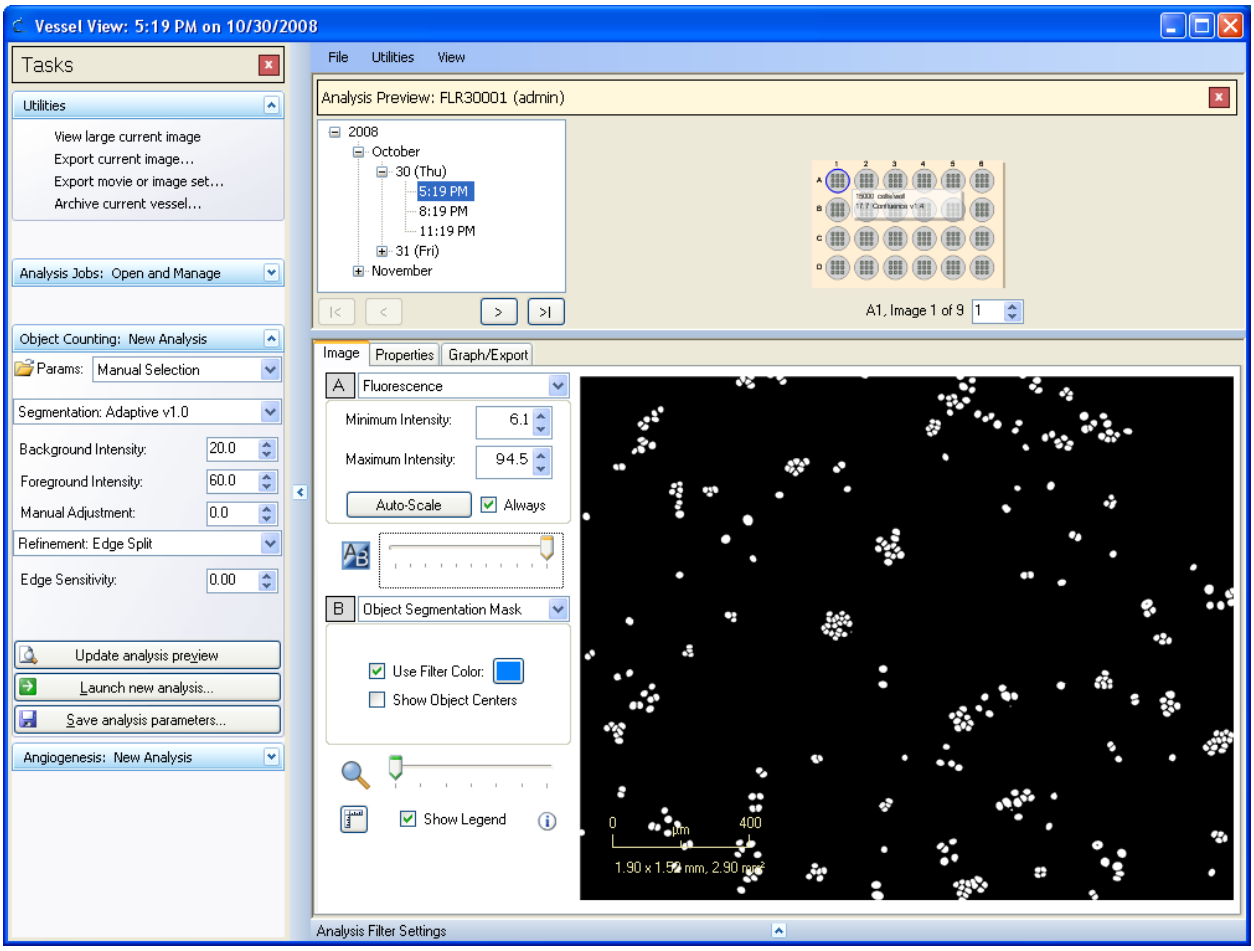


Figure 9-6. The Vessel View Window in Preview Mode.

The Banner at the top of the Vessel View Window is beige and it reads Analysis Preview. The Analysis Filter Settings Pane has been opened. Channel A is set to Fluorescence and Channel B is set to Object Segmentation Mask. In this figure, the Show Object Centers box is not checked

Object Segmentation Mask

There are two check boxes available when Object Segmentation Mask is selected in the A or B Channel pull-down window:

Use Filter color

Check this box if you want a color to be applied to all Objects that will be excluded from analysis via the Filter settings. To select a specific color for the Filter Color, click directly on the colored box (this box is set to blue in Figure 9-6). See Section 9.3.8, Filter Settings, page 224 for further information on applying Filter settings.

Show Object Centers

Check this box to show the center of each Object. The center of each Object will be indicated with a red "X." When you first launch Analysis Preview Mode, this

box will be checked. However, if you uncheck the Show Object Centers box, the centers will not be shown the next time Preview Mode is launched.

Analysis Filter Settings

Segmentation and Refinement Parameters identify potentially relevant Objects within an image area. In Figure 9-6, the Segmentation Mask has identified all the Objects that meet the criteria for inclusion as defined by their fluorescence intensities and proximities. However, the User might choose to use additional criteria to eliminate some of the Objects from graphical analysis and/or exported data. This subsequent filtration of the data is performed using the Analysis Filter Settings in the Analysis Filter Settings Pane.

The Analysis Filter Settings Pane can only be opened during Preview Mode or after an Analysis Job has been opened. Analysis Filter Settings will be more fully discussed in Section 9.3.6, page 222.

Unapplied Analysis Preview Mode

When Analysis Preview Mode is first opened, the image will reflect the analysis results of whatever Segmentation and Refinement Parameter settings were present at the time the preview was launched. However, it's possible that after some initial evaluation, the User decides to adjust these settings. Changing any of the Segmentation or Refinement settings will shift the User into Unapplied Analysis Preview Mode. The Banner at the top of the Vessel View window will change from beige to yellow, and the text will read Unapplied Analysis Preview. This Banner change is a warning to the User that Segmentation and/or Refinement Parameters have been changed, but they have not yet been applied to the image. To apply the newly selected parameters, press the Update Analysis Preview button. The Image and graphical data will be updated to reflect the new settings, and the User will be returned to Analysis Preview Mode.

9.3.5 Launch New Analysis

Once the appropriate Segmentation and Refinement Parameters have been selected, it's time to launch an Analysis Job. A new analysis can be launched from Analysis Preview Mode or from Standard Mode by pressing the Launch New Analysis button in the Vessel View Tasks Pane. A new window will open. See

Figure 9-7.

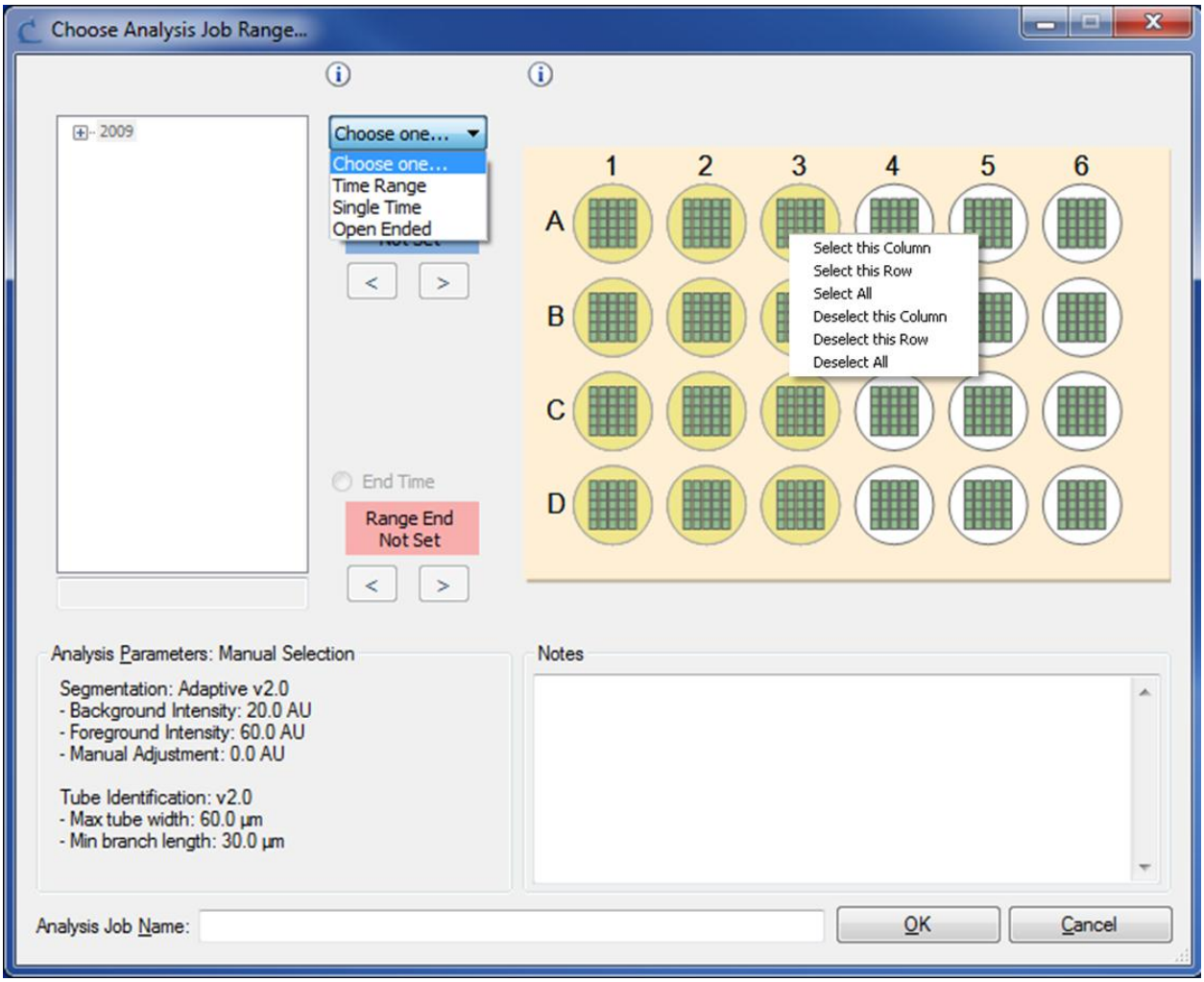


Figure 9-7. Launching a New Analysis

Preparing to Launch

Press the Launch New Analysis button and make the appropriate selections in the drop down selection window. The options include: a specified time range, a single time point, or an open ended job. An open ended job signifies that the experiment is currently ongoing and that you want each subsequent image in the sequence to be automatically analyzed per your job settings. This allows automated processing of images in real time so that one big job does not need to be completed at the end of the experiment. However, it is critically important that users continue to qualify their segmentations masks in subsequent images to verify that the analysis parameters completely represent the fluorescence observed in the images.

Select Spatial and Temporal Range of the Analysis Job

The Job Range window allows the User to select the temporal and spatial ranges that the final analysis will encompass.

Select Temporal Range of the Analysis

Use the Time-Tree to select the desired scan times.

The analysis must include a consecutive sequence of scans.

Select Spatial Range of the Analysis

Use the mouse and vessel image to select all desired wells or sectors. All images within a particular well or sector will be included in the analysis. Wells/sectors selected for analysis will be highlighted yellow. In

Figure 9-7, the wells in columns 1, 2 and 3 have been selected. Wells in columns 4,5,6 will NOT be included in the analysis.

- Regions of the vessel can be selected by clicking and dragging with the mouse.
- Individual wells or sectors can be selected by individually clicking on them with mouse.
- Right-click over in a scanned vessel/sector to activate the Selection/Deselection menu.
- Choose an option from the menu to select/deselect individual rows, columns or all.
- Previously selected wells/sectors can be individually deselected by clicking on them with the mouse.

Notes

Enter any notes here. These notes are NOT read-only – this is in contrast to the notes entered in the properties box associated with a scanned vessel. Therefore, it is possible for the original author or any other person to modify or delete these notes at any time, even after the analysis has been completed.

Name the Analysis

Give your Analysis Job a name. After the Analysis has been launched, it will appear under the Analysis Jobs: Open and Manage Task Bar in the Vessel View Tasks Pane.

Analysis Parameters

A summary of the Segmentation and Refinement Analysis Parameters that will be applied to the analysis are displayed along with the name of the Parameter set in the Job Range window. In Figure 9-6, the parameters were entered under the Manual Selection option. However, if saved Parameters were being applied, the name of the saved Parameters would be displayed here.

It is important to remember that the Segmentation and Refinement Analysis Parameters CANNOT be adjusted once an Analysis Job has been launched/completed.

Launch an Analysis Job

Press the OK button at the bottom of the Job Range window, and the job will be launched. Several things will happen when a new Analysis Job is launched:

The Job Name and Job Type will appear under the Analysis Jobs: Open and Manage Task Bar. An icon will appear to the left of the job name indicating that the job is still incomplete. See the Example job in Figure 9-8.

A new, Job Processor window will open indicating the status of the analysis. This window will indicate the number of images processed and the number of images remaining.

The IncuCyte™ controller will join the analysis when it is available (for instance, if it is not scanning).

When an Analysis Job is launched, the computer used to launch the job will participate in processing the data. However, the corresponding IncuCyte™ controller can also participate in processing. The Job Processor window reflects processing taking place on the local PC, but there is no window that reflects the status of controller-based processing.

Join an Analysis

It is frequently possible to increase the rate of processing by joining an ongoing Analysis Job. See Figure 9-8. The Open and Manage Jobs window is displayed as it will appear under two different circumstances.

On the left, a completed job (Paclitaxel) has been selected. At the bottom of the window, the analysis type is indicated (Object Counting Analysis) as well as the User (admin) who launched the analysis. The time and date at which the job was completed are also indicated.

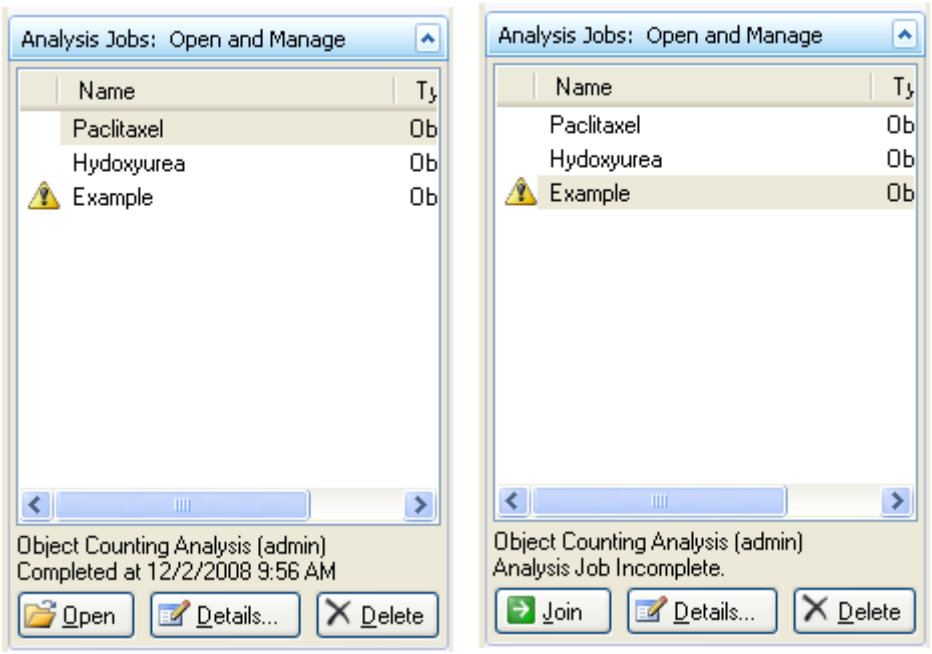


Figure 9-8. Join an Analysis

The buttons at the bottom of the window give the User the options of opening the job, viewing the job details or deleting the job.

IMPORTANT: Once a job has been deleted, it is gone forever. It CANNOT be restored. So be judicious about deleting jobs.

On the right, a job (Example) has been selected that is currently being processed. The incomplete status of this job is indicated by the yellow "construction" icon to the left of the job name. The analysis type and User are listed at the bottom of the window. However, there is no date of completion available since processing is ongoing. Instead, the window indicates that the Analysis Job is incomplete. Additionally, the button that read Open on the left now reads Join. If the computer participating in the analysis has a multi-core processor, then the Analysis Job can be joined by that same computer. Press the Join button, and a second Processor Window will open. If the computer participating in the processing has a multiple core processor, then multiple Processor Windows can be open at any given time (one for each core) in order to expedite the rate of processing. Although it is still possible to continue joining (and open more Processor Windows), we recommend against opening more windows than the total number of cores. Opening an excessive number of Processor Windows can actually decrease the rate of processing.

Ongoing Analysis Jobs can also be joined by other computers that have the IncuCyte™ FLR software. To join the analysis on a separate computer, launch the IncuCyte™ software, locate the vessel, open the Vessel View window, select the ongoing analysis and then press the Join button.

While joining an ongoing Analysis Job will generally increase the rate at which processing occurs, at some point excessive joining will result in diminishing returns because the IncuCyte™ controller will be overwhelmed. Therefore, it is important to monitor the progress of job processing. If multiple computers are working on a single job, and the rate of processing decreases, then reduce the amount of joined processing. This same principle applies to excessive joining on a single computer.

Exiting and Rejoining an Analysis Job

An ongoing analysis can be exited by closing all the open Processor Windows. Exiting an Analysis Job will suspend participation of the local PC in an ongoing analysis. Remember, however, that even though your PC is no longer involved in processing, the controller will continue to process the Analysis Job on its own. If the analysis is still incomplete at a later time (because the controller hasn't completed it yet), it can be rejoined by any PC by selecting the job and pressing the Join button once again.

Closing the IncuCyte™ software will NOT automatically exit an ongoing Analysis Job. Even after the software has been closed, the Processor Windows will remain open, and the PC will continue processing the data. If the Processor Window(s) are closed by the User, the controller will continue processing the data.

It is possible to have multiple incomplete Analysis Jobs listed in the Open and Manage Jobs window that are not being actively processed by any PC. In this case, the controller will continue to process the jobs in the order in which they were added. Once the controller has completed the first previously incomplete Analysis Job, it will move on to the next until all pending jobs are fully processed.

In Summary, Analysis Jobs can be exited by:

Closing all the Processor Windows

Logging off of your computer

Shutting down your computer

Termination of an Ongoing Analysis Job

The ONLY way to fully terminate an ongoing Analysis Job, including Open Ended Jobs, is to select the job in the Open and Manage Jobs window and then press the Delete button. See Figure 9-8. All processing will cease, and the Analysis Job will no longer appear in the window. If any Processing Windows were open at the time the analysis was terminated, they will indicate that the job was completed. Close these leftover windows.

Exiting vs. Terminating Ongoing Analysis Jobs:

Exit an ongoing Analysis Job by closing any open Processor Windows. Exiting an ongoing analysis will suspend processing by the PC; however, processing will continue on the IncuCyte™ controller. The Job will remain in the Manage Jobs Window with a "construction" icon indicating that the Job is still incomplete.

Terminate an ongoing Analysis Job by pressing the Delete button below the Open and Manage Jobs Task Bar. Pressing the Delete button will terminate all processing on all PCs and also on the controller. The Analysis Job will be removed from the Manage Jobs window.

Analysis Job Errors

If an Analysis Job was completed with errors, then the name of Job will appear under the Analysis Jobs: Open and Manage Task bar with an icon indicating that error(s) occurred during the processing. Information regarding the nature of the processing errors can be viewed by pressing the Details button at the bottom of the window. See Figure 9-9. Jobs that were completed with errors can still be opened, but keep in mind that the job contains errors.

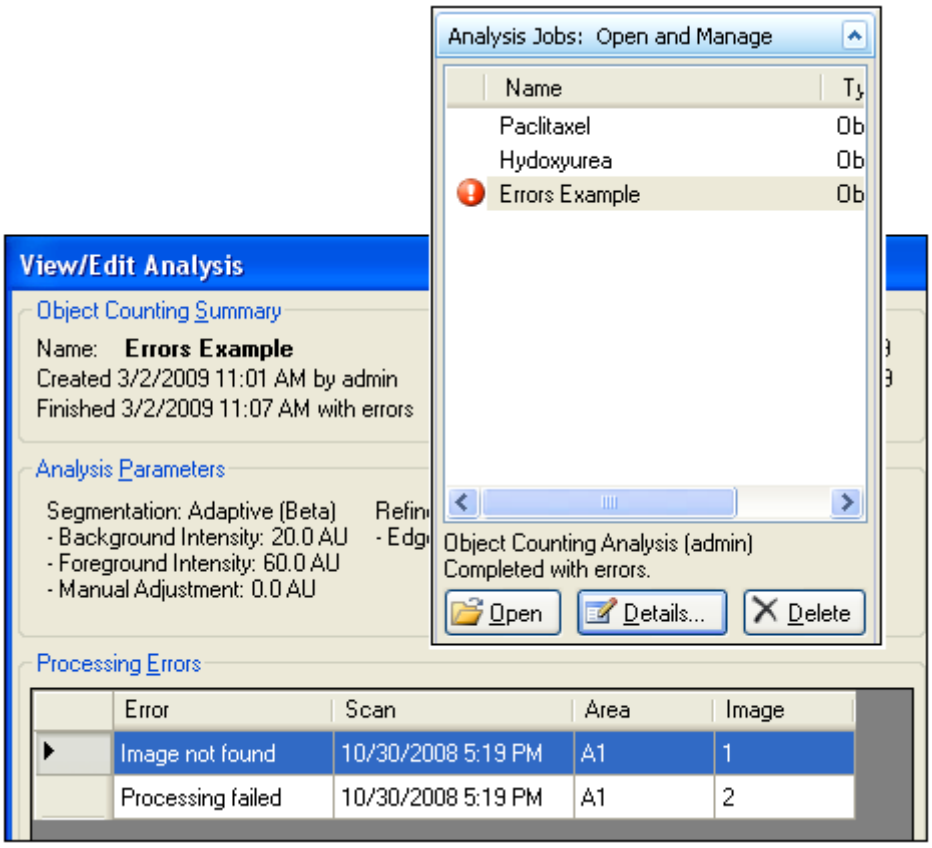


Figure 9-9. Analysis Job with Errors

9.3.6 Open and Manage Analysis Jobs

Completed Analysis Jobs will appear in the window under the Analysis Jobs: Open and Manage Tasks Bars. To open a job, press the Open button at the bottom of the window. Note that only completed Analysis Jobs can be opened. Ongoing jobs can only be joined.

Open an Analysis Job

Pressing the Open button will load the job. Opening a completed Analysis Job will resemble launching an Analysis Preview. In both cases, the Object Segmentation Mask will become available under the A and B Channel Settings, and Object Properties can be displayed. Additionally, the Analysis Filter Settings Tasks Pane will become active and can be opened. However, the completed Analysis Job is different in several respects:

- A green banner appears at the top of the Vessel View window, and it displays the job name.
- Every image of every scan listed in the Time Tree has been processed according to the selected Segmentation and Refinement Parameters. Therefore, the User is free to jump between different images and different scan times, and in all cases the images will be fully processed.
- The Vessel Image at the top of the Vessel View window will only indicate a scan pattern for those wells/sectors that were included in the analysis. Wells/sectors that were not included in the analysis cannot be selected.
- Time plots can be created and data for all images and time points can be exported.
- The Open and Delete buttons become inactive. It is not possible to delete a job that is currently open.

View Analysis Jobs

To view a history of all analysis jobs run on a specific IncuCyte™, click on the Scan drop-down menu at the top of the main user interface window and choose View Analysis Jobs. A new window will appear showing all analysis jobs and related information (date created, date completed, label, cell type, *etc*.) about each job (Figure 9-10). Double-click on a job to bring up that analysis.

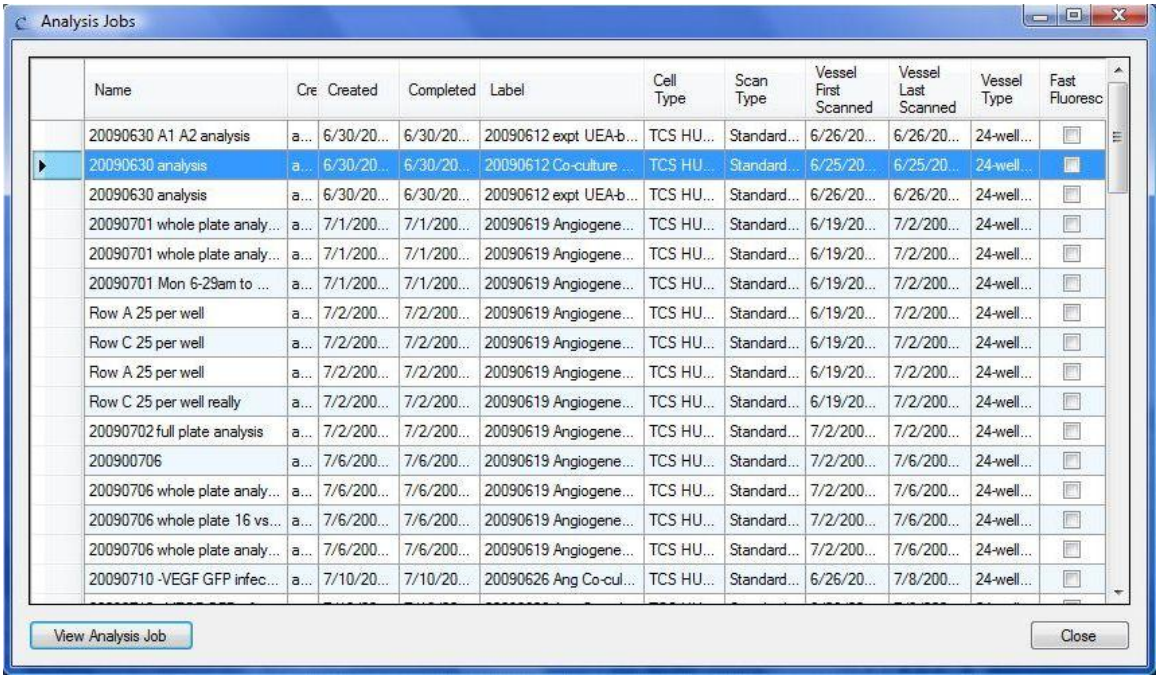


Figure 9-10. The Analysis Jobs window.

Analysis Job Details

Press the Details button to see the Analysis Parameters and other information associated with the job. Add or remove any relevant Notes. The nature of any errors that occurred during processing will be displayed here.

The Details button is active whenever a completed or ongoing job is selected in the Tasks Pane. It is not exclusively available after a job has been opened.

Close an Analysis Job

Close the job by clicking the red “X” at the far left of the green banner, closing the Vessel View window, opening a new job, or launching a new preview or a new Analysis Job.

Delete an Analysis Job

To delete an Analysis Job, select it in the Tasks Pane and then press the Delete button. Keep in mind that processing Analysis Jobs can be time consuming and that deleted jobs cannot be restored. So, be cautious when selecting a job for deletion.

Analysis Jobs and Archived Scans

When scans/vessels are archived, any Analysis Jobs that were affiliated with those vessels are archived along with the vessel. These jobs can be opened and manipulated as would be the case for a non-archived vessel. The data for the archived Analysis Job will no longer take up valuable controller disk space. All the same, the data will still have to be stored in an alternate location.

9.3.7 The Object Counting Analysis Filter Pull-Down Menu

The Analysis Filter pull-down menu is similar to the Parameters pull-down menu under the Object Counting: New Analysis Task Bar (The Parameters Pull-Down Menu, page 208).

The Object Counting Analysis Filter pull-down menu is displayed in Figure 9-11. It is currently set to Manual Selection.

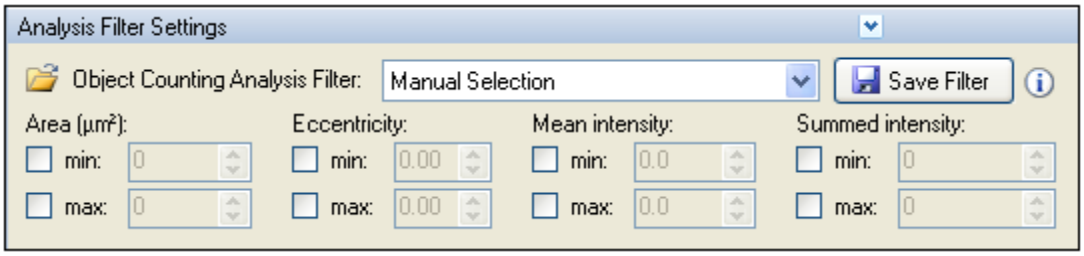


Figure 9-11. The Object Counting Analysis Pull-Down Window

Initially, two options will be available under the Analysis Filter pull-down menu: Manual Selection and Unfiltered. These two options will ALWAYS be available.

After one or more Filter Settings has been saved, these alternate Filter Settings will also be available in the pull-down menu. Selecting saved Filter Settings will populate the corresponding Object Property value(s), and the settings will automatically be applied to all images for all scan times included in the Analysis Job.

The Manage Analysis Filter Settings option will become available after at least one Filter Setting has been saved. Use this selection to rename or delete Filter Settings.

New Filter Settings can only be selected when the Analysis Filter pull-down menu is set to Manual Selection. Adjusting any of the Filter Metrics when saved Filter Settings are open will cause the pull-down menu to revert to Manual Selection.

Use the Save Filter button to save selected Filter Settings.

9.3.8 Filter Settings

Once an image has been processed, either via the Preview function or as part of a complete Analysis Job, all Objects will have Object Property values. These values can be visualized by holding the mouse over an Object in the Object Segmentation Mask.

Object Properties: Definitions

Area:

Total area of an Object (μm^2)

Eccentricity:

A measure of how round/compact the Object is. The values for Eccentricity range from 0 to 1, with a circle having a value of 1

Mean Intensity:

The average fluorescent intensity within an Object

Summed Intensity:

The sum of the intensities of the pixels in the Object

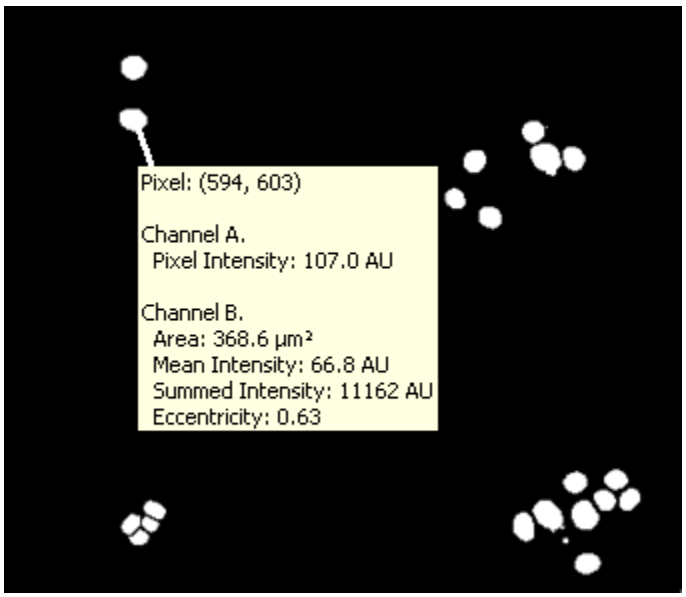


Figure 9-12. View the Processed Values Associated with an Object

See Figure 9-12. The coordinates for the selected Pixel will always be displayed. Which additional values will be displayed depends on the current selections in the A and B Channels. In Figure 9-12, Channel A has been set to Fluorescence and Channel B has been set to Object Segmentation Mask. When a Channel is set to Fluorescence, the only value associated with the image (aside from the coordinates) is the Pixel Intensity. So for the Channel A component of the display, only the value for Pixel Intensity is indicated. When a Channel is set to Segmentation Mask, then all values for all Object Properties are available. Therefore, the value for each Object Property for the selected Object is indicated in the Channel B component of the display. Review values for multiple Objects within the image to determine which values would be most appropriate with regard to adjusting Filter Settings.

Any Objects that are excluded from the image as a result of adjusting the Filter Settings can be identified with a color.

In Figure 9-13, the minimum Object Area has been set to 200µm^2, and the Filter Color has been set to blue. Therefore, all Objects in the image with an area of less than 200µm^2 are colored blue. All blue Objects have been excluded from analysis.

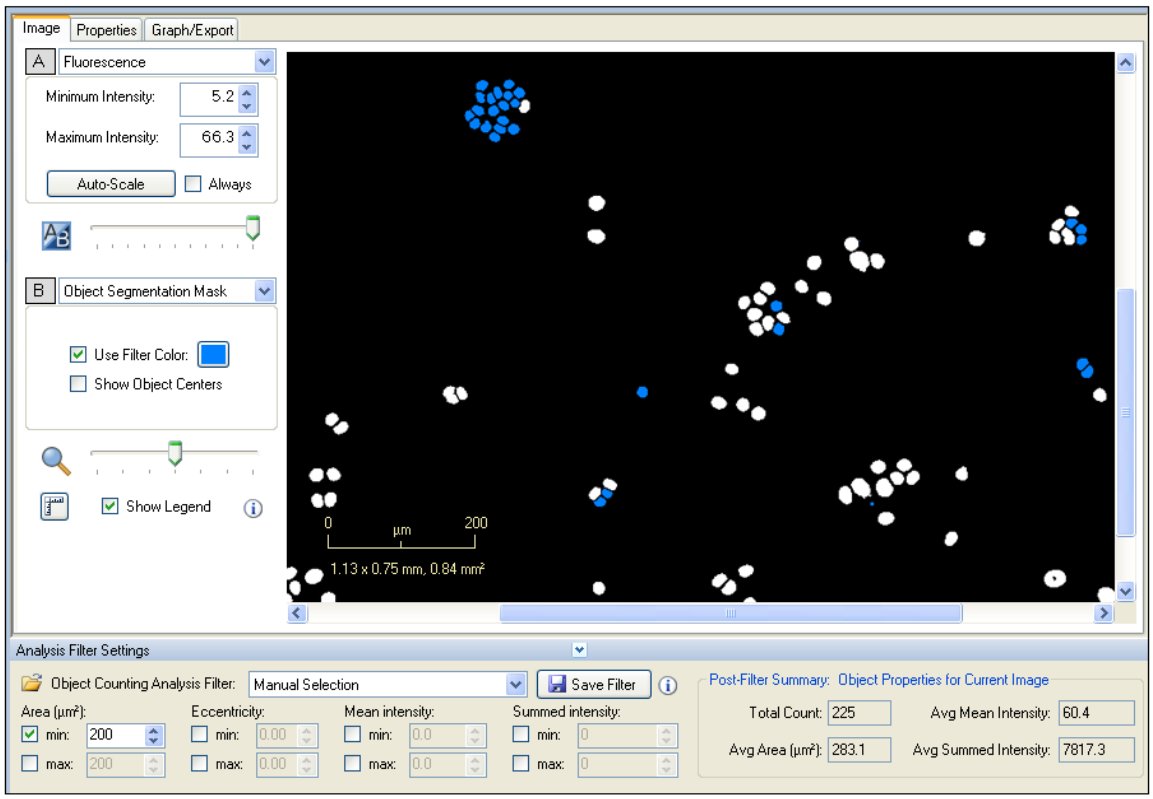


Figure 9-13. The Analysis Filter Settings Task Pane. Adjust settings to exclude Objects with Minimum Area of less than 200µm^2.

The exclusion of these Objects is reflected not just in the image but also:

In the Post-Filter Summary in the Vessel View window. The values in the Post-Filter Summary will change to reflect changes in the Filter Settings. In Figure 9-13, the Total Count reads 225. Prior to setting the minimum Object Area to 200µm^2, the Total Count was 338.

The Metric data under the Graph/Export tab.

When Filter Settings are adjusted, they are initially applied ONLY to the currently selected image. This is because a certain degree of processing is required to apply the new settings to the image. The time required to reprocess a single image to reflect updated Filter Settings is quite short. The time required to reprocess an entire Analysis Job is more substantial. Thus, in the interest of time, Filter Settings are initially applied to one image at a time in order to facilitate ease of manipulation. This is the case both for images that are being previewed and also images from completed Analysis Jobs. However, there are some important differences to keep in mind.

Adjust Filter Settings in Analysis Preview Mode

Filter Settings in Preview Mode apply only to the currently selected image. However, if a different image is selected, the User will be exited from Preview

Mode, and the Segmentation and Refinement Parameters as well as Filter Settings must be reset for the new image.

Adjust Filter Settings on a Completed Analysis Job

When Filter Settings are adjusted within a completed Analysis Job, the settings will be applied only to the currently selected image. However, if a new image is selected, the Filter Settings will automatically be applied to the new image as well. Additionally, the software will "remember" the results from the previous image. Thus, the reprocessed data from each consecutively selected image will be added and temporarily stored. All the same, any images that have NOT yet been selected will not reflect the adjusted Filter Settings. The software warns the User that Filter Settings have been applied to only a subset of the data by changing the vessel image color to red and displaying a corresponding message at the top of the Vessel View window. See Figure 9-14. In this example, Image 1 from Well A1 is the only image that has been selected. If you navigate to the

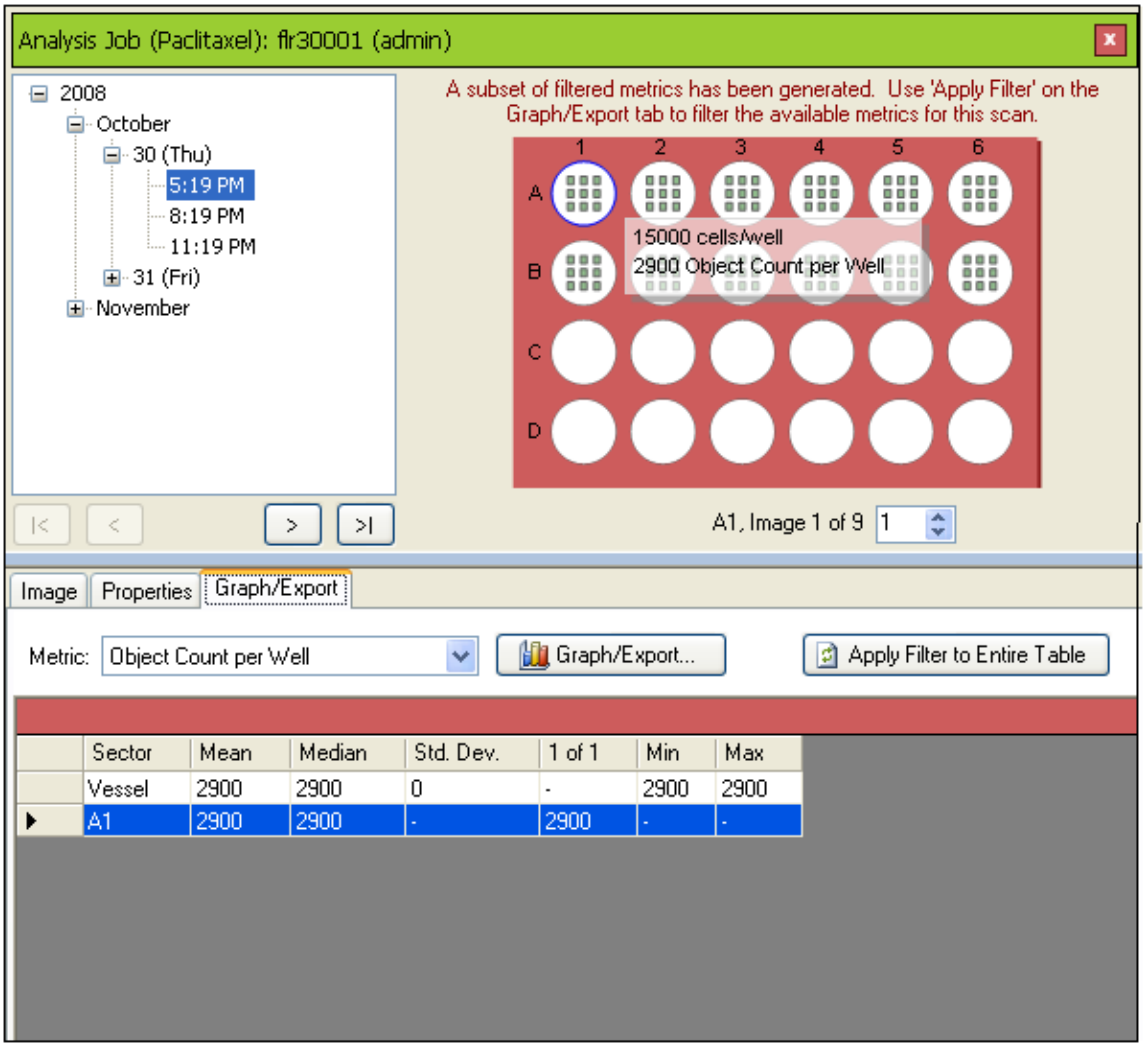


Figure 9-14. Filter Settings Applied to a Subset of the Data. Note the green banner at the top of the Vessel View window indicating that a completed Analysis Job has been opened.

Graph/Export tab, it will be very clear that for the Metric: Object Count per Well, data only exist for this single image. To apply the selected Filter Settings to additional images, select them individually. As the images are selected, the data table in the Graph/Export tab will be expanded to reflect the addition of new data. To apply the settings to all images simultaneously, push the button: Apply Filter to Entire Table.

Note that the warning in Figure 9-14 will only appear if Filter Settings are adjusted AND the selected Metric is specific to job analysis. For instance, the default Metric will always be Confluence (even if an Analysis Job has been opened). Because adjusting the Filter Settings does not affect the Confluence Metric, no warning will appear until a Metric has been selected that is affected by adjusting the Filter Settings.

Saving Analysis Filter Settings

The User may define Filter Settings that will be applicable to multiple experiments. To save Filter Settings, press the Save Filter button and give the settings a name. The saved settings will now be available under the Object Counting Analysis Filter pull-down menu. Saved Filter Settings can be deleted or renamed using the Manage Analysis Filters option, also available under the Object Counting Analysis Filter pull-down menu.

Users are encouraged to save all Analysis Filter Settings that they anticipate applying on a regular basis. Every time a new Analysis Job is launched, any saved Analysis Filter Settings will automatically be processed with the new job. Obviously, all possible saved Filter Settings will not automatically be applied when the job is opened; however, if any saved Filter Settings are subsequently selected in the Object Counting Analysis Filter pull-down menu, then all images included in the job will already have been post-processed with the selected Filter Settings. Thus, application of the settings will require no additional processing and the entire data set can be more easily and rapidly manipulated. Export and graphing functions will also be expedited.

Pin to Job

When saved Analysis Filter Settings are opened, the Save Filter button becomes the Pin to Job button. "Pinning" expresses a preference for a particular Filter Setting for a particular Analysis Job. If Filter Settings have been Pinned to a job, they will automatically be selected when a job is opened, and the Pin to Job button will be inactive.

Pinning is a time-saving measure. Because the settings have been pinned, all images in the Analysis Job have already been post-processed to include the specific filter preferences. Therefore, the data can be graphed and exported more rapidly.

Although any saved Filter Settings can be applied to any completed Analysis Job, only one set of Filter Settings can be Pinned to any one Analysis Job at any given time.

How To Pin

To Pin to a Job, select and open a completed Analysis Job. Then select the desired Filter Settings and press the Pin to Job button. Only saved filters can be pinned, and filters can only be pinned to jobs that are currently open.

How to "Unpin"

To "Unpin" a saved Filter Setting from an Analysis Job, you can Pin a different saved Filter Setting instead. Alternatively, select the Unfiltered option from the Filter Settings pull-down menu, and then Pin the Unfiltered selection to the job. Now, no filters will be applied to that job.

Delete or Rename Analysis Filter Settings

Use the Manage Analysis Filters option under the Analysis Filter pull-down menu to delete or rename previously saved Filter Settings.

> Recall that when an Analysis Job is launched ALL saved Analysis Filter Settings are automatically applied to that job for User convenience. Therefore, deleting a saved Filter Setting will potentially affect other users who are under the assumption that the setting is still saved and will be applied when they launch a new job.

Delete Filter Settings that Have been Pinned

All saved Filter Settings can be selected and deleted. Deleted Filter Settings will no longer be available under the Analysis Filter pull-down menu UNLESS they were previously pinned to a job. Filter Settings that have been pinned to a job will remain intact, and they will remain pinned to the job until they are specifically unpinned from that job. See Figure 9-15. In this figure, the setting Example

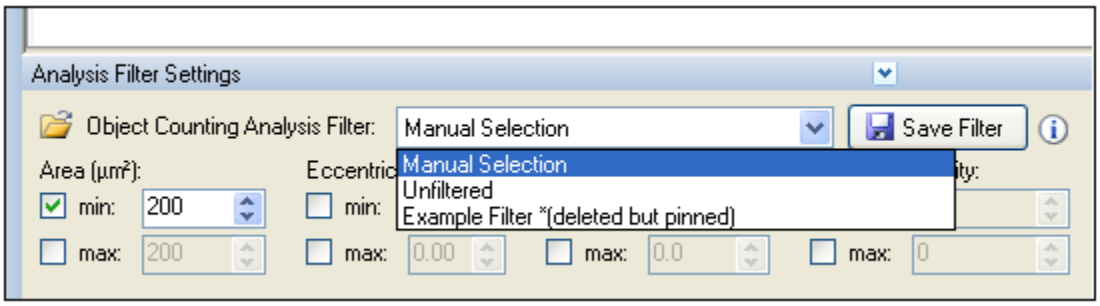


Figure 9-15. Deleted Filter Still Pinned to Job

Filter has been deleted. However, subsequent to deleting the Example Filter setting, a job was opened to which this setting had previously been pinned. Now, when the job is opened the saved Example Filter is still available; however, the fact it that was deleted is indicated in parenthesis. If Example Filter is no longer desired, it can be unpinned. However, after it has been unpinned from this job, it will no longer be available in the pull-down menu.

9.3.9 Graph/Export Data from Fluorescent Scans (Unprocessed Vessels)

Data from fluorescent scans can only be graphed/exported from the Vessel View window. Select the Graph/Export tab in the Vessel View window, and then press the Graph/Export button. For unprocessed vessels, this Graph/Export window will be the same as described for non-fluorescent scans (Section 8.10.2, page 170) except that one additional Metric will be available:

Fluorescence Image Mean

The mean of all the Pixel Intensity values within a given area of a fluorescently scanned image

Export of fluorescence scan graph/data is the same as export of non-fluorescent graph/data.

Graph/Export Time Plots of Processed Jobs

After processing an Analysis Job, new Metrics will be available for graphing and export. See the Essen BioScience Object Counting Application Note for more information regarding these Metrics. Open the Vessel View window, and press the Graph/Export button to open the corresponding window. See Figure 9-16. Note that Custom Region is also available for graph/export of processed Analysis Jobs.

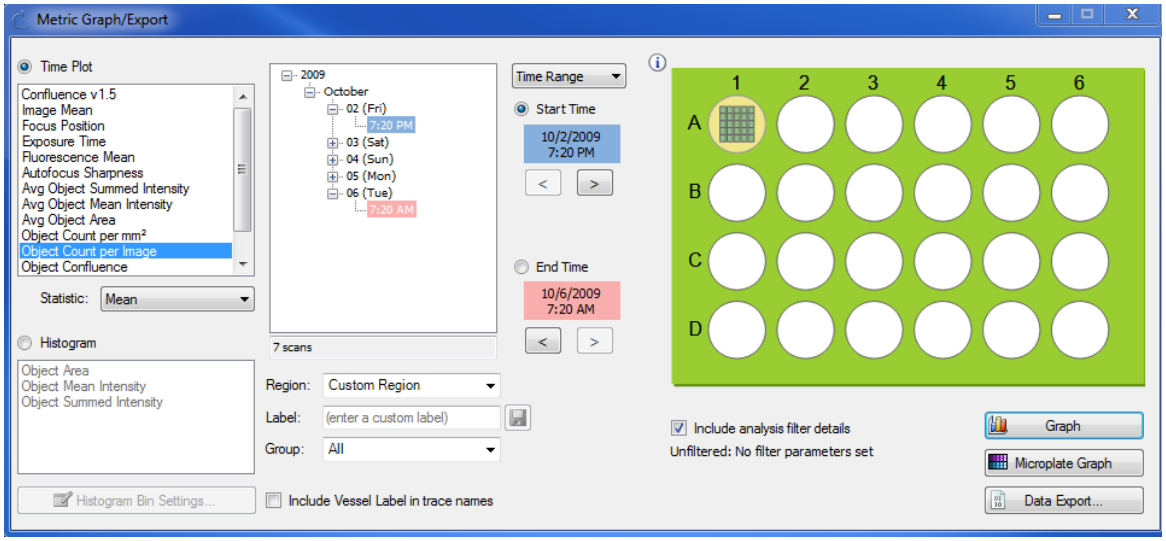


Figure 9-16. Graphing Data from Processed Jobs

To generate a Time Plot, choose Time Range and then select the desired Start and End Times. Select images for graphing and export as described for non-fluorescent data (Section 8.10.2, 170). The fluorescence Graph/Export window also lists any Filter Settings that are currently applied to the data. In Figure 9-16, only one Filter Setting has been applied (Area >= 50µm^2). To include the Filter Details with the graph or exported data, select the Include Filter Details check box located above the Graph button.

The Metrics available for the Object Counting Application are as follows:

Average Object Area

The average area for all Objects within an image

Average Object Mean Intensity

The average Mean Intensity for all Objects within an image

Average Object Summed Intensity

The average Summed Intensity for all Objects within an image

Object Count per Image

The total number of Objects within an image

Object Count per mm^2

The total number of Objects within an image divided by the image area in mm^2

Object Count per well

The average Object count per mm^2 times the area of the well in mm^2

Object Summed Intensity per mm^2

The total Summed Intensity of all objects within an image divided by the image area in mm^2

Export of fluorescence scan graph/data is the same as export of non-fluorescent graph/data.

The FLR Time Plot View Menu

Two options are available in the FLR Time-Plot window View pull-down menu:

Error Bars

Toggle Error bars on/off. If Error Bars are selected, choose between Standard Error or Standard Deviation

Show Analysis Filter

Choose whether to display any Analysis Filter Settings in the Graph window

Graph/Export Histograms of Processed Jobs

It is possible to generate histograms from Object Counting data. Histograms can only be generated for a single time point, so select Single Time instead of Time Range and then select the Histogram radio button. If the Histogram radio button is selected without first choosing Single Time, the selection will automatically be reset from Time Range to Single Time.

The Histogram window is now active. Three Metrics are currently available for Histograms:

Object Area (μm^2)

Object Mean Intensity

Object Summed Intensity

These same Metrics are available for Time Plots, except that in the case of Time Plots, the plot represents average values over time. Histograms display the distribution of the Metric at a single time point over the designated spatial grouping. As for Time Plots, Histogram spatial distribution can be anything from a single image to every scanned image.

To customize your Histogram, press the Histogram Bin Settings button. A new window will open allowing the User to adjust the Min/Max Values of the x-axis as well as the Bin Count. The software will assign recommended settings based

upon the data, but it is possible to substitute Custom values as desired. After Bin Settings have been selected, press the Graph button. Pressing the Graph button directly will generate a Histogram even if Bin settings are not viewed/adjusted first. The Histogram will simply use the settings assigned by the software. Increasing the number of Bins results in finer resolution of the distribution across the selected Metric. See Figure 9-17. In this example, a histogram has been generated that displays the distribution of Object Area in Well A1. Note that it is possible to Drag and Drop both the Histogram Data and Image. Because the Include Filter Details box was checked, the Filter Settings is also displayed on the graph.

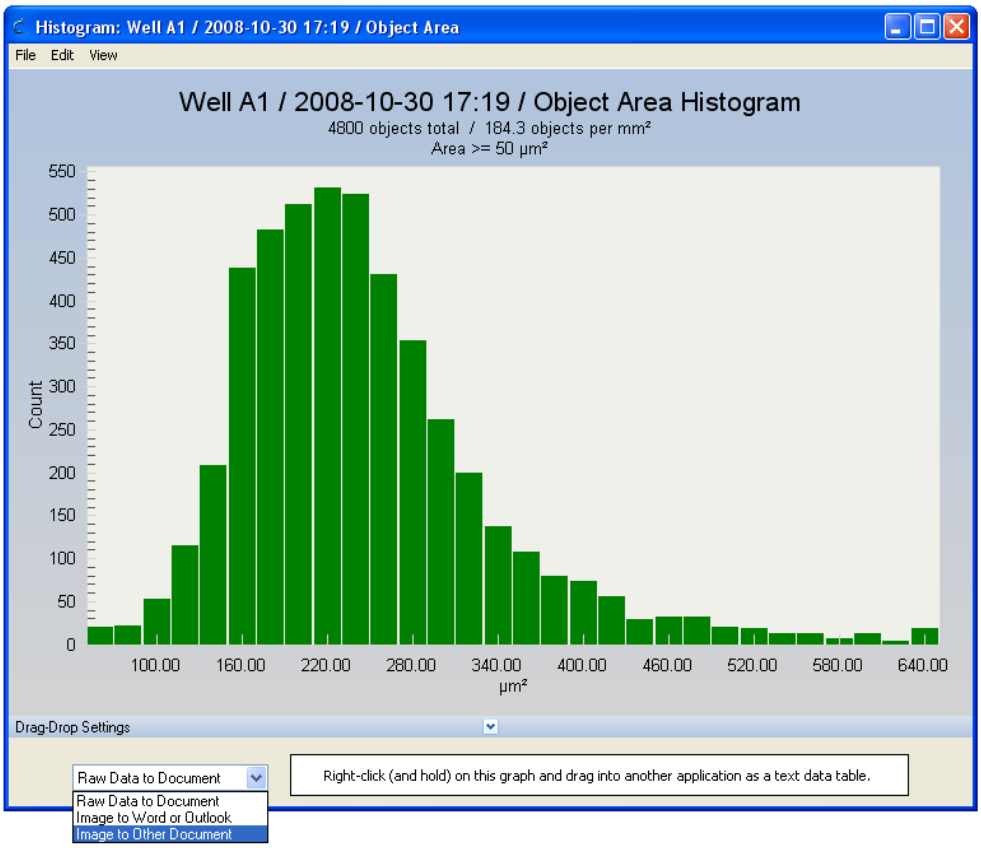


Figure 9-17. Object Area Histogram

Export of fluorescence scan graph/data is the same as export of non-fluorescent graph/data.

The FLR Histogram View Pull-Down Menu

Two options are available in the FLR Histogram window View pull-down menu:

Y-Axis Display

Select Count or Percent for the Y-Axis Display. In Figure 9-17, the Y-Axis displays Count.

Show Analysis Filter

Choose whether to display any Analysis Filter Settings in the Graph window.

10 Getting Optimum Performance from your IncuCyte™

The IncuCyte™ is a phase contrast microscope and as such requires an unobstructed path to the specimen from both above and below. Keep in mind the following warnings to avoid image degrading problems.

- Keep vessels clean
- Avoid condensation
- Avoid bubbles
- Avoid media hanging on top of flasks
- Don't scratch vessels
- Avoid writing labels on the tops of vessels
- Avoid touching the tops and bottoms of vessels. Handle them from the side as much as possible.

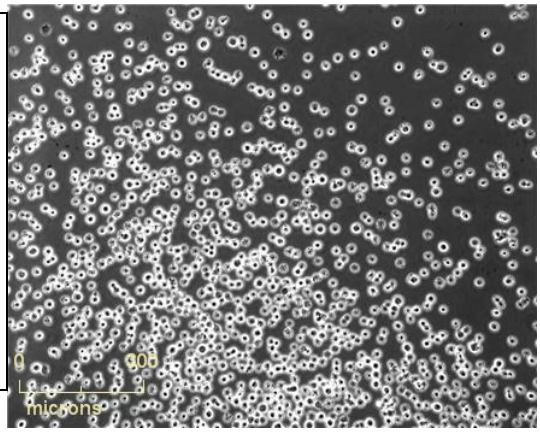
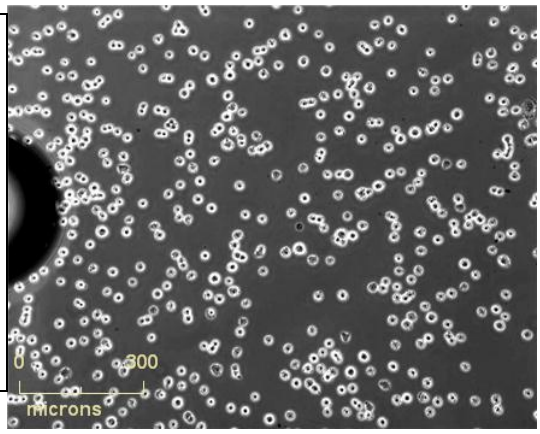
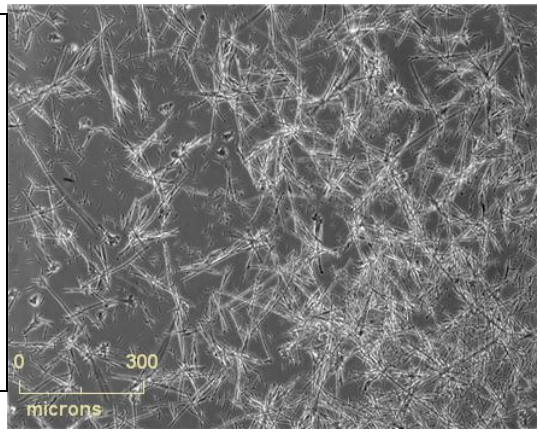
Vibration is another factor that can negatively affect image quality. The incubator that contains your IncuCyte™ should be on a sturdy stand. If possible, try to schedule scans at times when the incubator will not be accessed, as opening and closing the incubator door can cause image blurring even on a sturdy stand. Although it is desirable to not have large gaps in data collection, it is not necessary that the sampling be perfectly uniform. Scheduling daytime scans for just before work begins, at lunchtime, break times and again just after working hours may produce the best results. This also ensures that samples in the IncuCyte™ will be available during normal working hours in the lab.

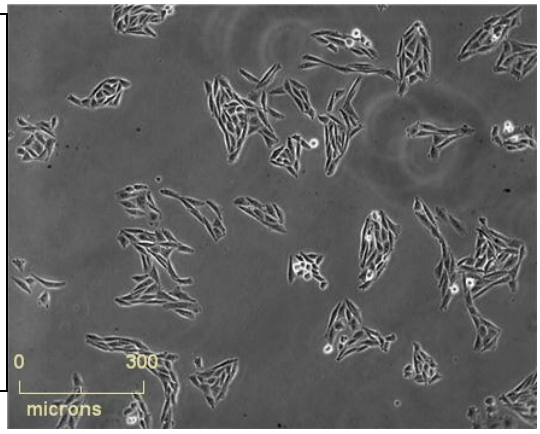
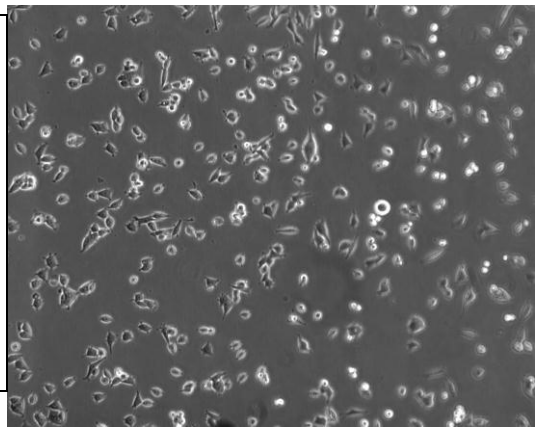
Another useful rule to follow is "don't over-scan your samples." We recommend keeping scan times under 45 minutes and duty cycles under 50%. As an example, 40 minute long scans scheduled every 3 hours results in a duty cycle of 22.2%. Under these conditions, any local temperature rises will typically be less than 1 degree C and relatively short lived.

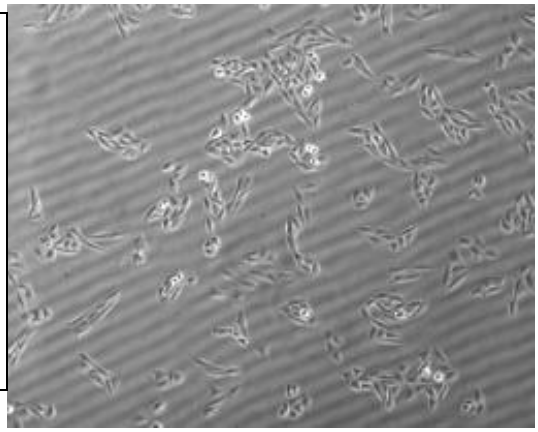
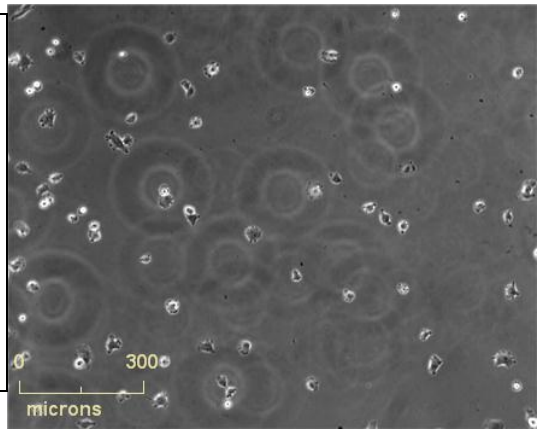
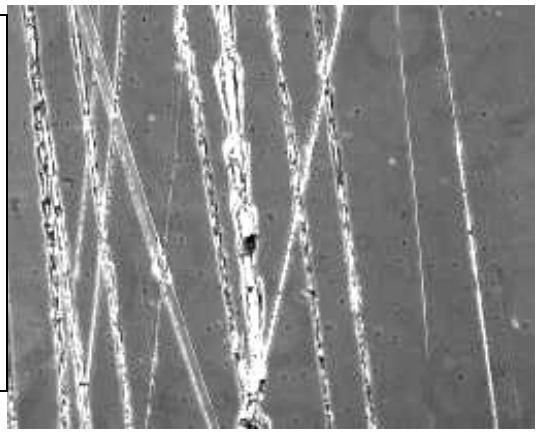
The IncuCyte™ is capable of collecting nearly 1500 images in 40 minutes, so another good reason to avoid such "over-scanning" is to limit the amount of data collected. This will keep hard drives from filling up unnecessarily and keep the system more responsive. We have found that between 0.5 and 1 images per square centimeter is usually sufficient to characterize the growth of cells in most tissue culture vessels. For example 48 images will usually characterize a T75 flask very well. With good technique that results in uniform seeding, even fewer images can be used. Also, with some slower growing cell types scanning every 4 or even 6 hours may be sufficient.

11 Troubleshooting

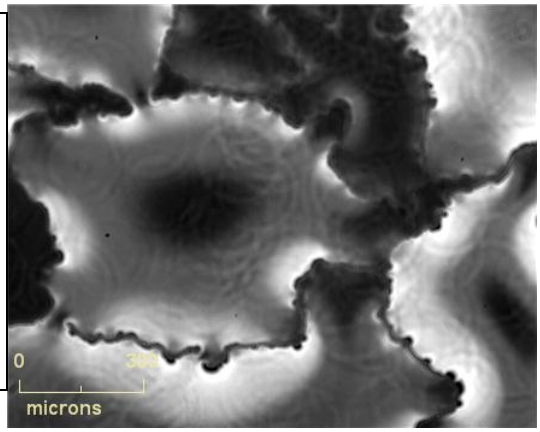
11.1 Images and What They Mean

Cells tend to clump together immediately after plating and before fully adhering to the surface. This problem is exacerbated by swirling the plates and/or placing freshly plated cells directly into the incubator. It is recommended that cells sit at room temperature until they are well enough seated to resist clumping. Then, put them into the incubator.	
Bubble on the bottom of the well on the far left side of the image. Cells appear rounded because they were just seeded and have not yet fully attached. Bubbles on the bottom of the well are distinguishable from floating bubbles because the cells will be clustered around the outside of a bubble on the bottom.	
Test compound has come out of solution and formed crystals in the media.	

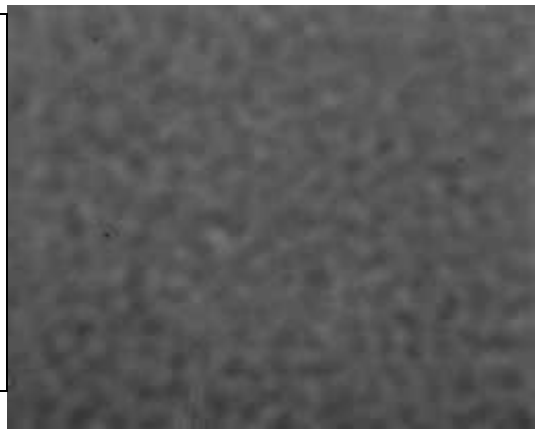
IncuCyte™ photographed condensation present on the outside, bottom of the plate instead of the cells. Be sure to avoid condensation on the insides and outsides of all vessels prior to placing them into the IncuCyte™.	
See top, right-hand corner. Bubbles on the surface of the media throw shadows onto the cells.	
The cells in the left portion of the image are in focus while the cells in the right portion are not. This is an indication that the flask is not seated flat in the tray.	

Striations are visible on the bottom of the well. This is an artifact of the way the microplate was created, and it is NOT related to IncuCyte™ functioning. Try switching to a different plate or maybe to a different brand of plates.	
Cells were scanned shortly after being seeded, and some cells are still floating (represented by large, out-of-focus circles).	
Severe scratches on the bottom of the flask.	

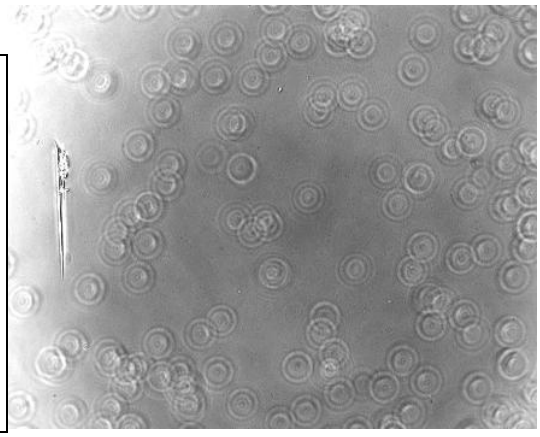
This is another example of condensation on the bottom of the wells. Out-of-focus cells are visible through the condensation.



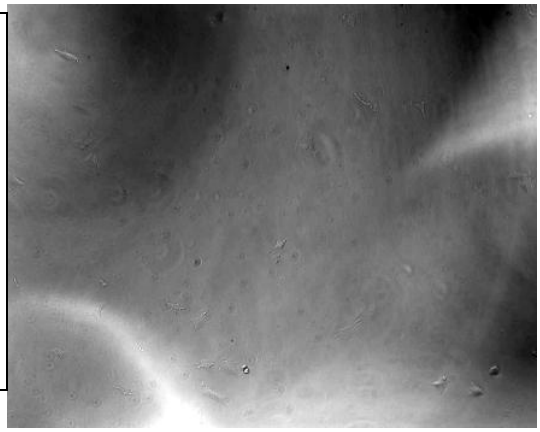
The flask was not seated properly, and the image is uniformly out of focus.



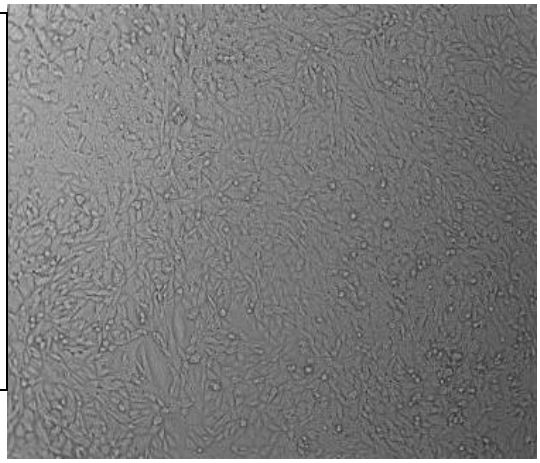
There is a scratch on the bottom of the well. The IncuCyte™ focused on the scratch instead of the cells, so that the scratch is in focus and the cells are out of focus.



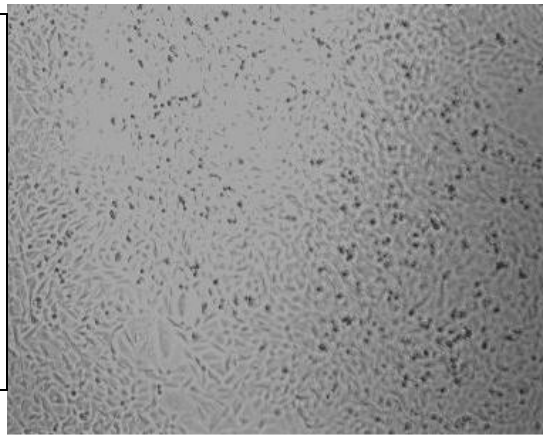
A large bubble is floating on the surface of the media creating a highly distorted image.



This image of confluent cells has virtually no contrast. A large droplet is probably suspended from the top of the vessel destroying the contrast. Alternatively, a meniscus is creating the problem.



Sometimes, in shallow cell culture vessels, the media can form a “bridge” between the top and bottom of the vessel. This bridge will act as a lens and destroy the image quality.



11.2 **Contacting Technical Support**

If you are experiencing technical difficulty with the IncuCyte or there is a “Device Error” in the status bar, follow the instructions below to export and email a log file to Essen BioScience technical support.

1. Log into the IncuCyte software GUI with an account that has administrator or user permissions as shown in Figure 11-1.
2. Click on “Administer IncuCyte” button on the left hand side of the software.
3. Click on the “Logs” tab on upper part of the screen.
4. Then click the “Export” button.

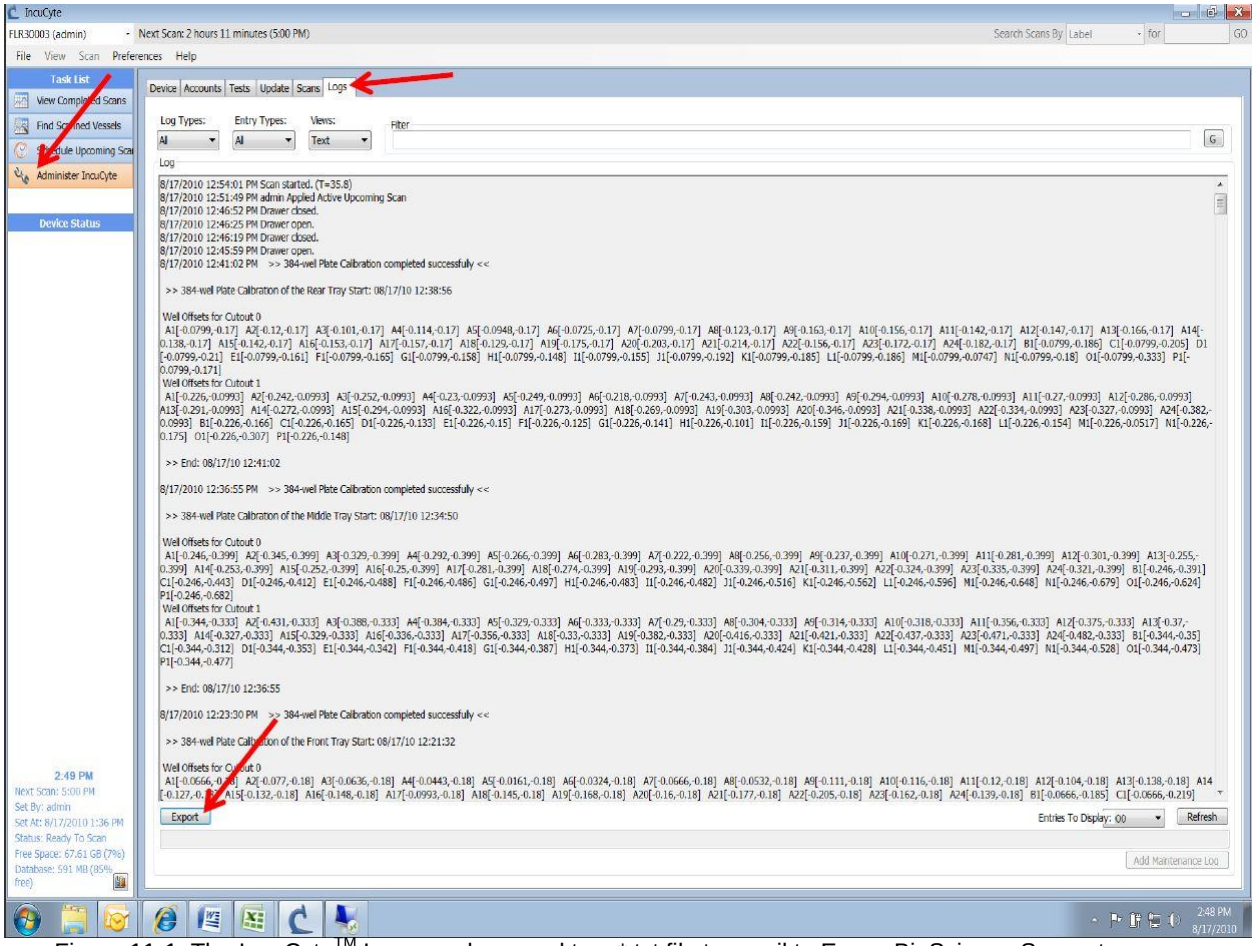

IncuCyte
Next Scan: 2 hours 11 minutes (5:00 PM)
File View Scan Preferences Help
Task List
View Completed Scans
Find Scanned Vessels
Administer IncuCyte
Device Status
Device Accounts Tests Update Scans Logs
Log Types: Entry Types: Views: Filter
Log
8/17/2010 12:54:01 PM Scan started. (T=35.8)
8/17/2010 12:51:49 PM admin Applied Active Upcoming Scan
8/17/2010 12:46:52 PM Drawer closed.
8/17/2010 12:46:25 PM Drawer open.
8/17/2010 12:46:19 PM Drawer closed.
8/17/2010 12:45:59 PM Drawer open.
8/17/2010 12:41:02 PM >> 384-well Plate Calibration completed successfuly <<
>> 384-well Plate Calibration of the Rear Tray Start: 08/17/10 12:38:56
Well Offsets for Cutout 0
Well Offsets for Cutout 1
>> End: 08/17/10 12:41:02
8/17/2010 12:36:55 PM >> 384-well Plate Calibration completed successfuly <<
>> 384-well Plate Calibration of the Middle Tray Start: 08/17/10 12:34:50
Well Offsets for Cutout 0
Well Offsets for Cutout 1
>> End: 08/17/10 12:36:55
8/17/2010 12:23:30 PM >> 384-well Plate Calibration completed successfuly <<
>> 384-well Plate Calibration of the Front Tray Start: 08/17/10 12:21:32
Well Offsets for Cutout 0
Export
Entries To Display: 00
Refresh
Add Maintenance Log
2:49 PM
Next Scan: 5:00 PM
Set By: admin
Set At: 8/17/2010 1:36 PM
Status: Ready To Scan
Free Space: 67.61 GB (7%)
Database: 591 MB (85% free)
2:48 PM
8/17/2010

Figure 11-1. The IncuCyte™ Logs can be saved to a *.txt file to email to Essen BioScience Support

5. Click “Yes” to export the entire logs file.

6. Name the text file after you company or entity.
7. Email the text file along with a brief description of the issue to local support. Local support can be found at www.essenbioscience.com/contactUs.html

12 Specifications

	IncuCyte ™	IncuCyte™ HD	IncuCyte™ FLR	IncuCyte™ EX
Operating Systems	Microsoft Windows® XP Microsoft Windows® Vista Windows® 7	Microsoft Windows® XP Microsoft Windows® Vista Windows® 7	Microsoft Windows® XP Microsoft Windows® Vista Windows® 7	Microsoft Windows® XP Microsoft Windows® Vista Windows® 7
Image Resolution	0.93µm 1280 x 1024 Pixels	0.93µm 1280 x 1024 Pixels	10X Objective : 1.49µm or 20X Objective : 0.74µm 1280 x 1024 Pixels	0.93µm 1280 x 1024 Pixels
Objective	20X	20X	20X or 10X	20X
Native Image Format	JPEG*	JPEG*	Phase : JPEG* Fluorescence : 16-bit PNG*	JPEG
Exported Image Format	JPEG, TIFF	JPEG, TIFF	JPEG, PNG, TIFF, RAW	JPEG, TIFF
Exported Movie Format	WMV, AVI, Metamorph®	WMV, AVI, Metamorph®	WMV, AVI, Metamorph®	WMV, AVI, Metamorph®
High Definition (HD) Optics	No	Yes	Yes	Optional
Image Size (typical)	150 Kbytes	400 Kbytes	Phase : 400 Kbytes Fluorescence : 1.1 Mbytes	150 Kbytes with HD: 400 Kbytes
Fluorescence Excitation/Emission	n/a	n/a	450-490 nm/500-530 nm	n/a
Internal Image Storage	500 Gbyte Hard Disk 2 Gbyte RAM	500 Gbyte Hard Disk 2 Gbyte RAM	>900 Gbyte Hard Disk 2 Gbyte RAM	>900 Gbyte Hard Disk 2 Gbyte RAM
Network Connection	Ethernet 10/100 Mbps RJ45 Connector	Ethernet 10/100 Mbps RJ45 Connector	Ethernet 10/100 Mbps RJ45 Connector	Ethernet 10/100 Mbps RJ45 Connector
Power	100-240 VAC 47-63Hz 2.0 Amps max @ 120V 1.0 Amps max @ 230V	100-240 VAC 47-63Hz 2.0 Amps max @ 120V 1.0 Amps max @ 230V	100-240 VAC 47-63Hz 2.0 Amps max @ 120V 1.0 Amps max @ 230V	100-240 VAC 47-63Hz 2.0 Amps max @ 120V 1.0 Amps max @ 230V
Controller Dimensions (H x W x D)	3.75" x 17.25" x 20.5" 9.5cm x 44cm x 52cm	3.75" x 17.25" x 20.5" 9.5cm x 44cm x 52cm	3.75" x 17.25" x 20.5" 9.5cm x 44cm x 52cm	3.75" x 17.25" x 20.5" 9.5cm x 44cm x 52cm
Microscope Dimensions (H x W x D)	8.8" x 17.7" x 18.3" 224mm x 450mm x 465mm	8.8" x 17.7" x 18.3" 224mm x 450mm x 465mm	9.9" x 17.7" x 18.3" 251mm x 450mm x 465mm	8.8" x 17.7" x 18.3" 224mm x 450mm x 465mm
Controller Weight	30 lbs 13.6 kg	30 lbs 13.6 kg	33 lbs 15.0 kg	33 lbs 15.0 kg
Microscope Weight	32.5 lbs 14.8 kg	32.5 lbs 14.8 kg	36 lbs 16.3 kg	37 lbs 16.8 kg
Operating Environmental Controller	0°C to 33°C 5% to 90% RH Non-Condensing	0°C to 33°C 5% to 90% RH Non-Condensing	0°C to 33°C 5% to 90% RH Non-Condensing	0°C to 33°C 5% to 90% RH Non-Condensing
Operating Environmental Microscope	0°C to 42°C 5% to 95% RH Non-Condensing	0°C to 42°C 5% to 95% RH Non-Condensing	0°C to 42°C 5% to 95% RH Non-Condensing	0°C to 33°C 5% to 90% RH Non-Condensing

*Other image formats are available via export functions in the software.
** Other wavelengths are available by special order. Please contact your sales representative.

Notes:
Choice of 10X or 20X objective is only an option on the IncuCyte™ FLR.
IncuCyte™ FLR is only available with HD optics.

13 IncuCyte™ Catalogue

Item	Catalogue Number
IncuCyte™ Microscope System: Includes Onsite Installation and Training, (#4362) Essen IncuCyte™ Controller Unit, (#4364) Essen IncuCyte™ Base Software, (#5025-0106) IncuCyte™ Calibration Tray, Customer must choose 6 additional IncuCyte™ Trays prior to purchase	4363
IncuCyte™ HD Microscope System: Includes Onsite Installation and Training, (#4362) Essen IncuCyte™ Controller Unit, (#4364) Essen IncuCyte™ Base Software, (#5025-0106) IncuCyte™ Calibration Tray, Customer must choose 6 additional IncuCyte™ Trays prior to purchase	4381
IncuCyte™ FLR Microscope System: Includes Onsite Installation and Training, (#4368) Essen IncuCyte™ FLR Controller Unit, (#4376) IncuCyte™ FLR Gantry, (#4364) Essen IncuCyte™ Base Software including Fluorescent Object Counting Software, (#5025-0106) IncuCyte™ Calibration Tray, (#1221-0275) IncuCyte™ Microslide and Calibration Tray, Customer must choose 6 additional IncuCyte™ Trays prior to purchase and must choose between the following 2 lens options :10x Objective (#5050-0109-00A) or 20x Objective (#5050-0110-00A)	4376
IncuCyte™ EX Microscope System for integration with TAP: Includes Training, (#4387) Essen IncuCyte™ EX Controller Unit, (#4364) Essen IncuCyte™ Base Software, Requires integration by TAP	4371
IncuCyte™ EX HD Microscope System for integration with TAP: Includes Training, (#4387) Essen IncuCyte™ EX Controller Unit, (#4364) Essen IncuCyte™ Base Software, Requires integration by TAP	4384
CellPlayer™ 96-Well Cell Migration/Invasion Assay Kit: Includes (#9500-4400) 96-well Cell Migration Software Application Module, The WoundMaker 96 Tool, (#5025-0191) WoundMaker 96-Well Rinse Boat Assembly, (#4379) Fifteen 96-well ImageLock Plates, and (#5025-0116) IncuCyte™ Micro Plate Tray, Certificate of Analysis; (1500-0078-A00) Two (2) Biocision BCS-147 96F CoolBox System Includes CoolBox Microplate System with CoolSink 96F; (1500-0079-A00)One (1) Biocision BCS-133 M30 CoolBox System Includes CoolBox 30 System with CoolRack M30; and, (1500-0080-A00)One (1) Biocision BCS-106 CoolSink 96F for 96-well flat-bottom plates. Extracellular Matrix used in the Invasion Assay must be ordered separately	4443
CellPlayer™ Angiogenesis Software Application Module	4411
IncuCyte™ Fluorescence Calibration Standards Kit	4380
IncuCyte™ Tray 1: Tray which holds up to four (T25) tissue culture flasks	1221-0239
IncuCyte™ Tray 4: Tray which holds up to one (T162) tissue culture flask	1221-0240
IncuCyte™ Tray 5: Tray which holds up to one (T150, T175) tissue culture flask	1221-0241
IncuCyte™ Tray 6: Tray which holds up to one (T150,182,185) tissue culture flask	1221-0242
IncuCyte™ Tray 7: Tray which holds up to one (T225) tissue culture flask	1221-0243
IncuCyte™ Tray 9: Tray which holds up to two (100mm Petri Dish) tissue culture dishes	1221-0244
IncuCyte Tray 10: Tray which holds up to two (T225, Large plate) tissue culture flasks or large plates	1221-0245
IncuCyte™ Tray 11: Tray which holds up to three (Large Petri) tissue culture flasks	1221-0246
IncuCyte™ Tray 12: Tray which holds up to three (T25) tissue culture flasks	1221-0247
IncuCyte™ Tray 13: Tray which holds up to three (T25) tissue culture flasks	1221-0248
IncuCyte™ Tray 14: Tray which holds up to two (T75) tissue culture flasks	1221-0249

IncuCyte™ Tray 15: Tray which holds up to two (T75) tissue culture flasks	1221-0250
IncuCyte™ Tray 17: Tray which holds up to two (T75) tissue culture flasks	1221-0340
IncuCyte™ Tray 18 Assembly: Tray which holds 3 ibidi slides and contains features to restrain ibidi apparatus tubing	5025-0169
IncuCyte™ Tray 19: Tray which holds up to four (60mm dish) tissue culture dishes	1221-0347
IncuCyte™ Tray 20: Tray which holds up to four (T25) tissue culture flasks	1221-0348
IncuCyte™ Tray 21: Tray which holds up to eight (35mm dish) tissue culture dishes	1221-0349
IncuCyte™ Micro Plate Tray: Tray which holds up to two tissue culture multi-well plates	5025-0116
IncuCyte™ Calibration Tray	5025-0106
IncuCyte™ Calibration and Microslides Tray: Tray which holds up to four tissue culture slides	1221-0275
50 pack of 24-Well ImageLock plates: 24-Well plates which facilitate precise, repeated imaging	4365
50 pack of 96 Well ImageLock plates: 96-Well plates which facilitate precise, repeated imaging	4379

14 Ordering Contact

www.essenbio.com

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15 Appendix – IncuCyte™ Supported Vessels

HD

Please note that all 384-well microplates and a subset of 96-well plates are only compatible with the IncuCyte™ HD system. 96 and 384-well plates have an extra column to the far right (HD?). If this column is checked, then the corresponding plates are ONLY compatible with an IncuCyte™ HD device.

25cm² Flasks:

Brand	Catalog #s	Flask Name	Tray Type
BD Falcon	353081	70ml Canted Neck Standard TC Phenolic Cap	12
	353082	70ml Canted Neck Standard TC Plug-seal Cap	12
	353109	70ml Canted Neck Standard TC Vented Cap	12
	354479	70ml Poly-D-Lysine 25 cm² Flask Plug-seal Cap	12
	354484	70ml Collagen I 25 cm² Flask Vented Cap	12
	354531	70ml Collagen I 25 cm² Flask Plug-seal Cap	12
	354532	70ml Fibronectin 25 cm² Flask Plug-seal Cap	12
	354533	70ml Laminin 25 cm² Flask Plug-seal Cap	12
	354534	70ml Collagen IV 25 cm² Flask Plug-seal Cap	12
	354536	70ml Poly-D-Lysine 25 cm² Flask Vented Cap	12
	356479	70ml Poly-D-Lysine 25 cm² Flask Plug-seal Cap	12
	356484	70ml Collagen I 25 cm² Flask Vented Cap	12
	356531	70ml Collagen I 25 cm² Flask Plug-seal Cap	12
	356536	70ml Poly-D-Lysine 25 cm² Flask Vented Cap	12
	353014	50ml Canted Neck Standard TC Plug-seal Cap	20
	353108	50ml Canted Neck Standard TC Vented Cap	20
Corning	3055	Triangular Angled Neck Cell Culture Flask with Phenolic-Style Cap	1
	3056	Triangular Angled Neck Cell Culture Flask with Vent Cap	1
	430168	Rectangular Canted Neck Cell Culture Flask with Plug Seal Cap	12
	430372	Rectangular Canted Neck Cell Culture Flask with Phenolic-Style Cap	12
	430639	Rectangular Canted Neck Cell Culture Flask with Vent Cap	12
Greiner	690160	50ml Canted Neck Standard TC Plug-seal Cap	20
Iwaki	3100-025	25cm² Tissue Culture Flask	12
Nunc	132703	Nunc EasYFlasks™ Polystyrene. Sterile, Angled Neck Filter Cap Poly-D-Lysine	13
	132706	Nunc EasYFlasks™ Polystyrene. Sterile, Angled Neck Filter Cap Collagen	13
	156340	Nunc EasYFlasks™ Polystyrene. Sterile, Angled Neck Vented Cap	13
	156367	Nunc EasYFlasks™ Polystyrene. Sterile, Filter Cap	13
	136196	Nunclon™Δ Canted Neck Standard TC Vented Filter Cap	20
	163371	Nunclon™Δ Canted Neck Standard TC Plug-seal Cap	20
TPP	90025	60ml Canted Neck Standard TC <<VENT>> Cap	13
	90026	60ml Canted Neck Standard TC Filter Cap	13

75cm² Flasks:

Brand	Catalog #s	Flask Name	Tray Type
BD Falcon	353023	250ml Straight Neck Standard TC Phenolic Cap	14
	353024	250ml Straight Neck Primaria TC Plug-seal Cap	14
	353110	250ml Straight Neck Standard TC Vented Cap	14
	353810	250ml Straight Neck PrimariaTC Vented Cap	14
	353824	250ml Straight Neck Primaria TC Plug-seal Cap	14
	353133	250ml Canted Neck Nontreated Plug-seal Cap	15
	353134	250ml Canted Neck Standard TC Phenolic Cap	15
	353135	250ml Canted Neck Standard TC Plug-seal Cap	15
	353136	250ml Canted Neck Standard TC Vented Cap	15
	354462	Collagen I 75 cm² Flask with plug-seal cap	15
	354485	Collagen I 75 cm² Flask with vented cap	15
	354488	Gelatin 75 cm² Flask with vented cap	15
	354521	Fibronectin 75 cm² Flask with plug-seal cap	15
	354523	Collagen IV 75 cm² Flask with plug-seal cap	15
	354524	Poly-D-Lysine 75 cm² Flask with plug-seal cap	15
	354537	Poly-D-Lysine 75 cm² Flask with vented cap	15
	354654	Gelatin 75 cm² Flask with plug-seal cap	15
	356462	Collagen I 75 cm² Flask with plug-seal cap	15
	356485	Collagen I 75 cm² Flask with vented cap	15

75 cm^2 Flasks:

Brand	Catalog #s	Flask Name	Tray Type
BD Falcon	356488	Gelatin 75 cm² Flask with vented cap	15
	356524	Poly-D-Lysine 75 cm² Flask with plug-seal cap	15
	356537	Poly-D-Lysine 75 cm² Flask with vented cap	15
	356654	Gelatin 75 cm² Flask with plug-seal cap	15
Corning	3275	Modified Triangular Straight Neck Cell Culture Flask with Phenolic-Style Cap	14
	3276	Modified Triangular Straight Neck Cell Culture Flask with Vent Cap	14
	430198	75cm^2 Flask	14
	430199	75cm^2 Flask	14
	3290	CellBIND® Rectangular Canted Neck Cell Culture Flask with Vent Cap	15
	3375	Rectangular Canted Neck Cell Culture Flask with Phenolic-Style Cap	15
	3376	Rectangular Canted Neck Cell Culture Flask with Vent Cap	15
	430641	Rectangular Canted Neck Cell Culture Flask with Vent Cap	15
	430720	Rectangular Canted Neck Cell Culture Flask with Plug Seal Cap	15
	430725	Rectangular Canted Neck Cell Culture Flask with Phenolic-Style Cap	15
Greiner	658170	250ml Canted Neck TC Flask	14
	658175	250ml Canted Neck TC Flask Filter Cap Lo-Profile	14
	658195	CellStar® T75 Flask, 250mL, ST, CN, Filter Cap 75 cm²	14
	658940	Poly-D Lysine Coated TC Flask	14
	658950	Collagen Type 1 Coated TC Flask	14
Iwaki	3110-075	75cm^2 Tissue Culture Flask	15
Nunc	153732	Nunclon™Δ Straight Neck Standard TC Plug-seal Cap	14
	178905	Nunclon™Δ Straight Neck Standard TC Vented Filter Cap	14
	132704	Nunc EasyFlask Poly-D-Lysine	17
	132707	Nunc EasyFlask Collagen I	17
	156472	Nunc EasyFlask Vent/Close Cap	17
	156499	Nunc EasyFlask Filter Cap	17
TPP	90075	75cm² Flask with Vent Cap	17
	90076	75cm² Flask with Filter Cap	17

150cm^2 Flasks:

Brand	Catalog #s	Flask Name	Tray Type
BD Falcon	354486	Collagen I 150 cm² Flask with vented cap	5
	354495	Poly-D-Lysine 150 cm² Flask with plug-seal	5
	354538	Poly-D-Lysine 150 cm² Flask with vented cap	5
	354645	Collagen I 150 cm² Flask with plug-seal cap	5
	354646	Fibronectin 150 cm² Flask with plug-seal cap	5
	355000	600mL Canted Neck Standard TC Vented Cap	5
	355001	600mL Canted Neck Standard TC Plug-seal Cap	5
	356486	Collagen I 150 cm² Flask with vented cap	5
	356495	Poly-D-Lysine 150 cm² Flask with plug-seal cap	5
	356538	Poly-D-Lysine 150 cm² Flask with vented cap	5
	356645	Collagen I 150 cm² Flask with vented cap	5
Corning	3291	CellBIND® 150cm² Rectangular Canted Neck Cell Culture Flask with Vent Cap	6
	430823	150cm² Rectangular Canted Neck Cell Culture Flask with Plug Seal Cap	6
	430824	150cm² Rectangular Canted Neck Cell Culture Flask with Phenolic-Style Cap	6
	430825	150cm² Rectangular Canted Neck Cell Culture Flask with Vent Cap	6
TPP	TP90150	690ml Cap	5
	TP90151	690mL Filter Cap	5

162cm^2 Flasks:

Brand	Catalog #s	Flask Name	Tray Type
Corning	3150	Traditional Straight Neck Cell Culture Flask with Phenolic-Style Cap	4
	3151	Traditional Straight Neck Cell Culture Flask with Vent Cap	4

175cm^2 Flasks:

Brand	Catalog #s	Flask Name	Tray Type
BD Falcon	353028	750ml Straight Neck Standard TC Plug-seal Cap	5
	353045	750ml Straight Neck Standard TC Phenolic Cap	5
	353112	750ml Straight Neck Standard TC Vent Cap	5
	353118	750ml Straight Neck Standard TC Vent Cap w barcode	5
	354478	Collagen I 175 cm² Flask with plug-seal cap	5

175cm^2 Flasks:

Brand	Catalog #s	Flask Name	Tray Type
BD Falcon	354487	Collagen I 175 cm² Flask with vented cap	5
	354526	Fibronectin 175 cm² Flask with plug-seal cap	5
	354528	Collagen IV 175 cm² Flask with plug-seal cap	5
	356478	Collagen I 175 cm², Flask with plug-seal cap	5
	356487	Collagen I 175 cm² Flask with vented cap	5
Corning	3292	CellBIND® Angled Neck Cell Culture Flask with Vent Cap	5
	431079	Angled Neck Cell Culture Flask with Plug Seal Cap	5
	431080	Angled Neck Cell Culture Flask with Vent Cap	5
	431085	Rectangular Angled Neck Cell Culture Flask with Phenolic Cap	5
	431306	Angled Neck Cell Culture Flask with Vent Cap and Bar Code	5
Nunc	132705	EasYFlasks™ Polystyrene. Sterile, Angled Neck Filter Cap, Poly-D-Lysine	5
	132708	EasYFlasks™ Polystyrene. Sterile, Angled Neck Filter Cap, Collagen I	5
	156502	Nunclon™Δ Polystyrene. Sterile, Straight Neck Vent Cap	5
	159910	EasYFlasks™ Nunclon™Δ Polystyrene. Sterile, Angled Neck Filter Cap	5
	159920	EasYFlasks™ Nunclon™Δ Polystyrene. Sterile, Angled Neck Vented Cap	5
	178883	Nunclon™Δ Polystyrene. Sterile, Straight Neck Filter Cap	5
	178983	Barcode Flasks Nunclon™Δ □	5

182cm^2 Flasks:

Brand	Catalog #s	Flask Name	Tray Type
Greiner	660160	CellStar T182 Flask, 550mL, TC, ST, LoPro, CN, Plug Cap 550 ML, 182 CM², STERILE	6
	660175	CellStar T182 Flask, 550mL, TC, ST, LoPro, CN, Filter Cap 550 ML,182 CM²	6
	661160	CellStar T182 Flask, 650mL, TC, ST, HiPro, CN, Plug Cap 650 ML, STERILE	6
	661175	CellStar T182 Flask, 650mL, TC, ST, HiPro, CN, Filter Cap 650 ML, STERILE	6
	661195	CellStar T182 Flask, 650mL, ST, HiPro, CN, Filter Cap	6
	661940	CellCoat T182 Flask, 650mL, w/PDL, ST, HiPro, CN, Plug Cap POLY-D-LYSINE	6
	661950	CellCoat T182 Flask, 650mL, w/PDL, ST, HiPro, CN, Plug Cap Collagen	6

185cm^2 Flasks:

Brand	Catalog #s	Flask Name	Tray Type
Nunc	144881	SoLo Flasks Nunclon™Δ Polystyrene. Sterile, Angled Neck, Vent Cap	6
	144903	SoLo Flasks Nunclon™Δ Polystyrene. Sterile, Angled Neck, Filter Cap	6

225cm^2 Flasks:

Brand	Catalog #s	Flask Name	Tray Type
BD Falcon	353138	Tissue Culture-Treated Flask; 75cm^2 with Canted Neck and vent cap	10
	353139	Tissue Culture-Treated Flask; 75cm^2 with Canted Neck and plug seal cap	10
Corning	3000	Flask Shape: Standard; Description: With Canted Necks, Phenolic Cap	7
	3001	Flask Shape: Standard; Description: With Canted Necks, Vent Cap	7
	431081	Corning® 225cm² Angled Neck Cell Culture Flask with Plug Seal Cap	10
	431082	Corning® 225cm² Angled Neck Cell Culture Flask with Vent Cap	10
Nunc	159933	Nunc EasYFlasks™ Nunclon™Δ, Angled Neck Vent/close Cap	10
	159934	Nunc EasYFlasks™ Nunclon™Δ, Angled Neck Filter Cap	10

500cm^2 Tripleflask:

Brand	Catalog #s	Flask Name	Tray Type
Nunc	132867	Nunc TripleFlasks™ Nunclon™Δ, Straigh Neck Vent/close Cap	5
	132913	Nunc TripleFlasks™ Nunclon™Δ, Straigh Filter Cap	5
	132920	Nunc TripleFlasks™ Nunclon™Δ, Straigh Filter Cap	5

84 cm^2 Autoflask

Brand	Catalog #s	Flask Name	Tray Type
Greiner	779160	AutoFlask™ Polystyrene TC-Treated for Adherent Cell Culture	Microplate

92.6cm^2 Roboflask:

Brand	Catalog #s	Flask Name	Tray Type
Corning	3059	Corning® RoboFlask™ Cell Culture Vessel for Manual Use, TCT, with Bar Code, No Septur	Microplate
	3067	CellBIND® Surface RoboFlask™ Cell Culture Vessel for Automation, Septum Cap	Microplate
	3068	CellBIND® Surface RoboFlask™ Cell Culture Vessel for Automation, Septum Cap	Microplate
	3069	Corning® RoboFlask™ Cell Culture Vessel for Automation, TCT, with Bar Code, Septum	Microplate
	3070	Corning® RoboFlask™ Cell Culture Vessel for Automation, TCT, with Bar Code, Septum Ca	Microplate
	3071	Corning® RoboFlask™ Cell Culture Vessel for Manual Use, TCT, with Bar Code, No Septur	Microplate

6-Well Plates:

Brand	Catalog #s	Plate Name	Tray Type
BD Falcon	353046	6 well Flat Bottom, Standard Tissue Culture Treated	Microplate
	353224	6 well Flat Bottom, Standard Tissue Culture Treated	Microplate
	353846	6 well Flate Bottom Primaria Tissue Culture Treated	Microplate
	353934	6 well Flat Bottom, Standard Tissue Culture Treated	Microplate
	354400	Collagen I 6-well Multiwell Plates	Microplate
	354402	Fibronectin 6-well Multiwell Plates	Microplate
	354404	Laminin 6-well Multiwell Plates	Microplate
	354413	Poly-D-Lysine 6-well Multiwell Plates	Microplate
	354417	6-well Multiwell Variety Pack	Microplate
	354428	Collagen IV 6-well Multiwell Plates	Microplate
	354431	6-well Multiwell Variety Pack	Microplate
	354432	Matrigel Matrix 6-well Multiwell Plates	Microplate
	354510	Matrigel Matrix 6-well Multiwell Plates for Hepatocytes	Microplate
	354515	Poly-L-Lysine 6-well Multiwell Plates	Microplate
	354595	Poly-D-Lysine/Laminin 6-well Multiwell Plates	Microplate
	354603	Matrigel Matrix Thin Layer 6-well Multiwell Plates	Microplate
	354652	Gelatin 6-well Multiwell Plates	Microplate
	354658	Poly-L-Ornithine/Laminin 6-well Multiwell Plates	Microplate
	356400	Collagen I 6-well Multiwell Plates	Microplate
	356413	Poly-D-Lysine 6-well Multiwell Plates	Microplate
	356515	Poly-L-Lysine 6-well Multiwell Plates	Microplate
	356652	Gelatin 6-well Multiwell Plates	Microplate
Corning	3335	Corning® CellBIND® 6 Well Microplate Flat Bottom, Standard Clear Plate, with Lid	Microplate
	3471	Costar® 6 Well Clear Flat Bottom Ultra Low Attachment Microplates, Stand w/Hydrogel	Microplate
	3506	Costar® 6 Well TC-Treated Microplates, Standard Clear Plate	Microplate
	3516	Costar® 6 Well TC-Treated Microplates, Standard Clear Plate	Microplate
Greiner	657160	CellStar, 6W Plate, TC, ST, FLT BOT, Lid WITH LID, SINGLE PACKED	Microplate
Nunc	140675	6 Well Plates Nunclon™Δ Sterile. Polystyrene, Individually wrapped	Microplate
	140685	6 Well Plates Nunclon™Δ Sterile. Polystyrene, 5 units/pack	Microplate
TPP	92006	6-Well TPP, Individually wrapped	Microplate
	92406	6-Well TPP, 4 per bag	Microplate

12-Well Plates:

Brand	Catalog #s	Plate Name	Tray Type
BD Falcon	353043	12 well Flat Bottom, Standard Tissue Culture Treated	Microplate
	353225	12 well Flat Bottom, Standard Tissue Culture Treated	Microplate
	354470	Poly-D-Lysine 12-well Multiwell Plates	Microplate
	354500	Collagen I 12-well Multiwell Plates	Microplate
	354501	Fibronectin 12-well Multiwell Plates	Microplate
	354502	Laminin 12-well Multiwell Plates	Microplate
	354503	Matrigel Matrix 12-well Multiwell Plates	Microplate
	356470	Poly-D-Lysine 12-well Multiwell Plates	Microplate
Corning	3336	Corning® CellBIND® 12 Well Microplate Flat Bottom, Standard Clear Plate, with Lid	Microplate
	3512	Costar® 12 Well TC-Treated Microplates, Standard Clear Plate	Microplate
	3513	Costar® 12 Well TC-Treated Microplates, Standard Clear Plate	Microplate
Nunc	150628	12 Well Plates Nunclon™Δ Sterile. Polystyrene	Microplate
TPP	92012	12-Well TPP, Individually wrapped	Microplate
	92412	12-Well TPP, 4 per bag	Microplate

24-Well Plates:

Brand	Catalog #s	Plate Name	Tray Type
BD Falcon	353047	24 well Flat Bottom, Standard Tissue Culture Treated	Microplate
	353226	24 well Flat Bottom, Standard Tissue Culture Treated	Microplate
	353847	24 well Flate Bottom Primaria Tissue Culture Treated	Microplate
	353935	24 well Flat Bottom, Standard Tissue Culture Treated	Microplate
	354408	Collagen I 24-well Multiwell Plates	Microplate
	354411	Fibronectin 24-well Multiwell Plates	Microplate
	354412	Laminin 24-well Multiwell Plates	Microplate
	354414	Poly-D-Lysine 24-well Multiwell Plates	Microplate
	354419	EHS Natrix 24-well Multiwell Plates	Microplate
	354433	Matrigel Matrix 24-well Multiwell Plates	Microplate
	354605	Matrigel Matrix Thin Layer 24-well Multiwell Plates	Microplate
	354619	Poly-D-Lysine/Laminin 24-well Multiwell Plates	Microplate
	354635	Growth Factor Reduced (GFR) Matrigel Matrix Thin Layer 24-well Multiwell Plates	Microplate
	354659	Poly-L-Ornithine/Laminin 24-well Multiwell Plates	Microplate
	356408	Collagen I 24-well Multiwell Plates	Microplate
	356414	Poly-D-Lysine 24-well Multiwell Plates	Microplate
Corning	3337	Corning® CellBIND® 24 Well Microplate Flat Bottom, Standard Clear Plate, with Lid	Microplate
	3473	Costar® 24 Well Clear Flat Bottom Ultra Low Attachment Microplates, Stand w/Hydrogel	Microplate
	3524	Costar® 24 Well TC-Treated Microplates, Standard Clear Plate	Microplate
	3526	Costar® 24 Well TC-Treated Microplates, Standard Clear Plate	Microplate
	3527	Costar® 24 Well TC-Treated Microplates, Standard Clear Plate	Microplate
Essen	4365	24-well Essen ImageLock Plate	Microplate
Greiner	662102	CellStar 24W Plate, PS, ST, FLT Bot, With Lid, Ind. Packed	Microplate
	662160	CellStar 24W Plate, PS,TC, ST, FLT Bot, With Lid, Ind. Packed	Microplate
	662165	CellStar 24W Plate, PS, TC, ST, FLT Bot, With Lid, 5 Units/bag	Microplate
Iwaki	5826-0024	24 Well Black Wall Glass based Culture Plate	Microplate
Nunc	142475	24 Well Plates Nunclon™Δ Sterile. Polystyrene, Individually wrapped	Microplate
	142485	24 Well Plates Nunclon™Δ Sterile. Polystyrene, 5 Units/pack	Microplate
Orange Scientific	5530300	24 Well Flat Test Plate, Polystyrene, TC-treated, individually wrapped	Microplate
	5530305	24 Well Flat Test Plate, Polystyrene, TC-treated	Microplate
SeaHorse	101037-004	24-well SeaHorse XF24 V7-PET	Microplate
TPP	92024	24-Well TPP, Individually wrapped	Microplate
	92424	24-Well TPP, 4 per bag	Microplate

48-Well Plates:

Brand	Catalog #s	Plate Name	Tray Type
BD Falcon	353078	48 well Flat Bottom, Standard Tissue Culture Treated	Microplate
	353230	48well Flat Bottom, Standard Tissue Culture Treated	Microplate
	354505	Collagen I 48-well Multiwell Plates	Microplate
	354506	Fibronectin 48-well Multiwell Plates	Microplate
	354507	Laminin 48-well Multiwell Plates	Microplate
	354508	Matrigel Matrix 48-well Multiwell Plates	Microplate
	354509	Poly-D-Lysine 48-well Multiwell Plates	Microplate
	356505	Collagen I 48-well Multiwell Plates	Microplate
	356509	Poly-D-Lysine 48-well Multiwell Plates	Microplate
Corning	3338	Corning® CellBIND® 48 Well Microplate Flat Bottom, Clear, with Lid, Sterile	Microplate
	3548	Costar® 48 Well TC-Treated Microplates, Standard Clear Plate	Microplate
Nunc	150687	48-Well Nunc Multidish, Individually wrapped	Microplate
	152640	48-Well Nunc Multidish, 5 per bag	Microplate

96-Well Plates:

Brand	Catalog #s	Plate Name	Tray Type	HD?
BD Falcon	353070	TCT 96-well Plate All Clear	Microplate	√
	353072	TCT 96-well Plate All Clear	Microplate	√
	353075	TCT 96-well Plate All Clear	Microplate	√
	353220	Black-Wall, Clear Bottom Optilux	Microplate	√
	353872	TCT 96-well Plate All Clear Primaria	Microplate	√
	353916	TCT 96-well Plate All Clear	Microplate	√
	353936	TCT 96-well Plate All Clear Ready Stack	Microplate	√
	353947	White-Wall, Clear Bottom Optilux	Microplate	√
	353948	Black-Wall, Clear Bottom Optilux	Microplate	√
	354407	Collagen I 96-well Microplates	Microplate	√
	354409	Fibronectin 96-well Microplates	Microplate	√

96-Well Plates:

Brand	Catalog #s	Plate Name	Tray Type	HD?
BD Falcon	354410	Laminin 96-well Microplates	Microplate	√
	354429	Collagen IV 96-well Microplates	Microplate	√
	354461	Poly-D-Lysine 96-well Microplates	Microplate	√
	354516	Poly-L-Lysine 96-well Microplates	Microplate	√
	354596	Poly-D-Lysine/Laminin 96-well Microplates	Microplate	√
	354607	Matrigel Matrix Thin-Layer Multiwell Assay Plate	Microplate	√
	354640	BD BioCoat™ Poly-D-Lysine 96-well Microplates, Black-Wall, Clear Bottom Optilux	Microplate	√
	354649	BD BioCoat™ Collagen I 96-well Microplates, Black-Wall, Clear Bottom Optilux	Microplate	√
	354650	BD BioCoat™ Collagen I 96-well Microplates, White-Wall, Clear Bottom Optilux	Microplate	√
	354651	BD BioCoat™ Poly-D-Lysine 96-well Microplates, White-Wall, Clear Bottom Optilux	Microplate	√
	354657	Poly-L-Ornithine/Laminin 96-well Microplates	Microplate	√
	354670	Laminin/Fibronectin 96-well Microplates	Microplate	√
	354689	Gelatin 96-well Microplates	Microplate	√
	356407	Collagen I 96-well Microplates	Microplate	√
	356461	Poly-D-Lysine 96-well Microplates	Microplate	√
	356516	Poly-L-Lysine 96-well Assay Plates	Microplate	√
	356519	BD BioCoat™ Collagen I 96-well Microplates, White-Wall, Clear Bottom Optilux	Microplate	√
	356640	BD BioCoat™ Poly-D-Lysine 96-well Microplates, Black-Wall, Clear Bottom Optilux	Microplate	√
	356649	BD BioCoat™ Collagen I 96-well Microplates, Black-Wall, Clear Bottom Optilux	Microplate	√
	356650	BD BioCoat™ Collagen I 96-well Microplates, White-Wall, Clear Bottom Optilux	Microplate	√
	356651	BD BioCoat™ Poly-D-Lysine 96-well Microplates, White-Wall, Clear Bottom Optilux	Microplate	√
	356689	Gelatin 96-well Microplates	Microplate	√
	356690	Poly-D-Lysine 96-well Microplates, clear	Microplate	√
	356692	BD BioCoat™ Poly-D-Lysine 96-well Microplates, Black-Wall, Clear Bottom Optilux	Microplate	√
	356693	BD BioCoat™ Poly-D-Lysine 96-well Microplates, White-Wall, Clear Bottom Optilux	Microplate	√
	356698	BD BioCoat™ Collagen I 96-well Microplates	Microplate	√
	356700	BD BioCoat™ Collagen I 96-well Microplates, Black-Wall, Clear Bottom Optilux	Microplate	√
Corning	3300	Corning® CellBIND® 96 Well Clear Flat Bottom Polystyrene Microplate, with Lid, Sterile	Microplate	√
	3340	Corning® CellBIND® 96 Well Clear Flat Bottom Black Polystyrene Microplate, with Lid, Ste	Microplate	√
	3372	Corning® CellBIND® 96 Well Clear Flat Bottom Black Polystyrene Poly-D-Lysine Microplat	Microplate	√
	3474	Corning® 96 Well Clear Flat Bottom Ultra Low Attachment Microplate	Microplate	√
	3585	Corning® 96 Well Clear Flat Bottom TC-Treated Microplate,with Lid, Sterile	Microplate	√
	3595	Corning® 96 Well Clear Flat Bottom TC-Treated Microplate, with Lid	Microplate	√
	3596	Corning® 96 Well Clear Flat Bottom Polystyrene TC-Treated Microplates	Microplate	√
	3598	Corning® 96 Well Clear Flat Bottom Polystyrene TC-Treated Microplates, with Lid	Microplate	√
	3599	Corning® 96 Well Clear Flat Bottom Polystyrene TC-Treated Microplates	Microplate	√
	3603	Corning® 96 Well Clear Flat Bottom TC-Treated Black Microplate Ind. wrapped ,Sterile	Microplate	√
	3610	Corning® 96 Well Clear Flat Bottom TC-Treated White Microplate Ind. wrapped ,Sterile	Microplate	√
	3628	Corning® 96 Well Clear Flat Bottom TC-Treated Microplate, with Lid,	Microplate	√
	3665	Corning® 96 Well Clear Flat Bottom Poly-D-Lysine Coated Microplate, with Lid	Microplate	√
	3666	Corning® 96 Well Clear Flat Bottom TC-Treated White Poly-D-Lysine Microplate Ind. wrapp	Microplate	√
	3667	Corning® CellBIND® 96 Well Clear Flat Bottom Black Polystyrene Poly-D-Lysine Microplat	Microplate	√
	3841	Corning® 96 Well Flat Clear Poly-D-Lysine, 20/Bag, with Lid, Sterile	Microplate	√
	3842	Corning® 96 Well Flat Clear Bottom Black Poly-D-Lysine, 20/Bag, with Lid, Sterile	Microplate	√
	3843	Corning® 96 Well Flat Clear Bottom White Poly-D-Lysine, 20/Bag, with Lid, Sterile	Microplate	√
	3903	Corning® 96 Well Flat Clear Bottom White Polystyrene TC-Treated Microplates, Sterile	Microplate	√
	3904	Corning® 96 Well Flat Clear Bottom Black Polystyrene TC-Treated Microplates, Sterile	Microplate	√
	3917	Corning® 96 Well Solid White Flat Bottom Polystyrene TC-Treated Microplate, with Lid	Microplate	√
	3997	Corning® 96 Well Clear Flat Bottom Polystyrene TC-Treated Microplates, with Lid	Microplate	√
Essen	4379	96-Well Essen ImageLock Plate	Microplate	√
Greiner	655087	CellStar, 96W Plate, PS, ST, TC, FLT BOT, BLK/uCLR 127,8/86/15 MM, TC,STERILE	Microplate	√
	655088	CellStar, 96W Plate, PS, ST, TC, FLT BOT, WHT/uCLR 127,8/86/15 MM, TC,STERILE	Microplate	√
	655090	CellStar, 96W Plate, PS, ST, TC, FLT BOT, BLK/uCLR, Lid 127,8/86/15 MM, TC, W.LID	Microplate	√
	655098	CellStar, 96W Plate, PS, ST, TC, FLT BOT, WHT/uCLR, Lid 127,8/86/15 MM, TC, W.LID	Microplate	√
	655160	PS, F-BOTTOM (chimney well), Crystal-Clear, Individual Packs	Microplate	√
	655162	PS, F-BOTTOM (chimney well),Crystal-Clear, 5 per Bag	Microplate	√
	655180	PS, F-Bottom (chimney well), Crystal-Clear, Sterile, Lid Condensation Rings,	Microplate	√
	655182	PS, F-Bottom (chimney well), Crysta-Clear, Lid Condensation Rings, 10 per Bag	Microplate	√
Iwaki	3860-096	96-Well Plate, 1 per Pack	Microplate	√
	3861-096	96-Well Plate, 10 per Pack	Microplate	√
Matrical	MGB101-1-1-LG	Black-Wall Glass Bottom MatriPlate 0.17mm	Microplate	√
	MGB096-1-2-LG-L	Black-Wall Glass Bottom MatriPlate 0.17mm with Lid	Microplate	√
	MGB096-1-2-LG-CC-L	Black-Wall Glass Bottom MatriPlate 0.17mm Collagen with Lid	Microplate	√
	MGB096-1-2-LG-PDL-L	Black-Wall Glass Bottom MatriPlate 0.17mm PDL with Lid	Microplate	√
	MGB096-1-2-LG-FN-L	Black-Wall Glass Bottom MatriPlate 0.17mm Fibronectin with Lid	Microplate	√
	MGB096-2-2-LG	White-Wall Glass Bottom MatriPlate 0.17mm	Microplate	√
	MGB096-2-2-LG-L	White-Wall Glass Bottom MatriPlate 0.17mm with Lid	Microplate	√
	MGB096-2-2-LG-CC-L	White-Wall Glass Bottom MatriPlate 0.17mm Collagen with Lid	Microplate	√

96-Well Plates:

Brand	Catalog #s	Plate Name	Tray Type	HD?
Matrical	MGB096-2-2-LG-PDL-L	White-Wall Glass Bottom MatriPlate 0.17mm PDL with Lid	Microplate	√
	MGB096-2-2-LG-FN-L	White-Wall Glass Bottom MatriPlate 0.17mm Fibronectin with Lid	Microplate	√
	MGB096-1-1-LG	Black-Wall Glass Bottom MatriPlate 0.72mm	Microplate	√
	MGB096-1-1-LG-L	Black-Wall Glass Bottom MatriPlate 0.72mm with Lid	Microplate	√
	MGB096-1-1-LG-CC-L	Black-Wall Glass Bottom MatriPlate 0.72mm Collagen with Lid	Microplate	√
	MGB096-1-1-LG-PDL-L	Black-Wall Glass Bottom MatriPlate 0.72mm PDL with Lid	Microplate	√
	MGB096-1-1-LG-FN-L	Black-Wall Glass Bottom MatriPlate 0.72mm Fibronectin with Lid	Microplate	√
	MGB096-2-2-LG	White-Wall Glass Bottom Matrical Plate 0.72mm	Microplate	√
	MGB096-2-2-LG-L	White-Wall Glass Bottom Matrical Plate 0.72mm with Lid	Microplate	√
	MGB096-2-1-LG-CC-L	White-Wall Glass Bottom Matrical Plate 0.72mm Collagen with Lid	Microplate	√
	MGB096-2-1-LG-PDL-L	White-Wall Glass Bottom Matrical Plate 0.72mm PDL with Lid	Microplate	√
	MGB096-2-1-LG-FN-L	White-Wall Glass Bottom Matrical Plate 0.72mm Fibronectin with Lid	Microplate	√
Perkin Elmer	6005181	ViewPlate-96, 96-well Microplate, White, Clear Bottom, Sterile, TC-treated with Lid 50/box	Microplate	√
	6005182	ViewPlate-96, 96-well Microplate, Black, Clear Bottom, Sterile, TC-treated with Lid 50/box	Microplate	√
	6005225	ViewPlate-96, 96-well Plate, Black, Clear Bottom, Sterile, TC-treated with Lid 2 x 25/box	Microplate	√
	6005263	ViewPlate-96, 96-well Microplate, White, Clear Bottom, Sterile, TC-treated with Lid 3/box	Microplate	√
	6005264	ViewPlate-96, 96-well Microplate, Black, Clear Bottom, Sterile, TC-treated with Lid 3/box	Microplate	√
Nunc	156545	96 MicroWell™ Plates Nunclon™Δ Sterile. Polystyrene	Microplate	√
	161093	96 MicroWell™ Plates Nunclon™Δ Sterile. Polystyrene	Microplate	√
	167008	96 MicroWell™ Plates Nunclon™Δ Sterile. Polystyrene	Microplate	√
	167311	96 Well Microplate Nunc Edge Nunclon-treated Sterile without lid	Microplate	√
	167314	96 Well Microplate Nunc Edge Nunclon-treated Sterile with lid	Microplate	√
	168055	96 MicroWell™ Plates Nunclon™Δ Sterile. Polystyrene	Microplate	√
	176747	96 MicroWell™ Plates CC3™, Clear, Polystyrene/Polymer Base	Microplate	√
	176748	96 MicroWell™ Plates CC3™, Black, Polystyrene/Polymer Base	Microplate	√
	176749	96 MicroWell™ Plates CC3™, White, Polystyrene/Polymer Base	Microplate	√
	267312	96 Well Microplate Nunc Edge Non-treated Nonsterile with lid	Microplate	√
	267313	96 Well Microplate Nunc Edge Non-treated Sterile with lid	Microplate	√
Sarstedt	83.1835.500	96-Well Plate. All Clear. Sterile with Lid	Microplate	√
TPP	92096	96-Well Plate. All Clear. Sterile with Lid, individually wrapped	Microplate	√
	92696	96-Well Plate. All Clear. Sterile with Lid, 5 Units/bag	Microplate	√

384-Well Plates:

Brand	Catalog #s	Plate Name	Tray Type	HD?
Aurora	31221	384-well Microplate, EB/SQ/ST/TR, Blk/Clr	Microplate	√
	31241	384-well Microplate, SQ/ST/TR, Blk/Clr	Microplate	√
	31421	384-well Microplate, EB/SQ/NS/TR, Blk/Clr	Microplate	√
	31441	384-well Microplate, SQ/NS/TR, Blk/Clr	Microplate	√
	31521	384-well Microplate, EB/SQ/NS/NT, Blk/Clr	Microplate	√
	31541	384-well Microplate, SQ/NS/NT, Blk/Clr	Microplate	√
	31621	384-well Microplate, EB/SQ/ST/TR, Wht/Clr	Microplate	√
	31641	384-well Microplate, SQ/ST/TR, Wht/Clr	Microplate	√
	31821	384-well Microplate, EB/SQ/NS/TR, Wht/Clr	Microplate	√
	31841	384-well Microplate, SQ/NS/TR, Wht/Clr	Microplate	√
	31921	384-well Microplate, EB/SQ/NS/NT, Wht/Clr	Microplate	√
	31941	384-well Microplate, SQ/NS/NT, Wht/Clr	Microplate	√
	32021	384-well Microplate, EB/SQ/ST/TR, All Clr	Microplate	√
	32041	384-well Microplate, SQ/ST/TR, All Clr	Microplate	√
	32221	384-well Microplate, EB/SQ/NS/TR, All Clr	Microplate	√
	32241	384-well Microplate, SQ/NS/TR, All Clr	Microplate	√
	32321	384-well Microplate, EB/SQ/NS/NT, All Clr	Microplate	√
	32341	384-well Microplate, SQ/NS/NT, All Clr	Microplate	√
	32451	384-well Aurora IQ-EB Ultra Low Base, EB/SQ/ST/TR, Blk/Clr	Microplate	√
	32651	384-well Aurora IQ-EB Ultra Low Base, EB/SQ/NS/TR, Blk/Clr	Microplate	√
	32751	384-well Aurora IQ-EB Ultra Low Base, EB/SQ/NS/NT, Blk/Clr	Microplate	√
BD Falcon	353961	384-well Microplates, tissue-culture treated, flat-bottom, with lid	Microplate	√
	353221	384-well Microplates, black/clear BD Optilux, TC-treated, sterile	Microplate	√
	353229	384-well Microplates, clear, TC-treated, with lid	Microplate	√
	353270	384-well Microplates, black/clear, BD Optilux, TC-treated, sterile, with lid	Microplate	√
	353274	384-well Microplates, black/clear, BD Optilux, TC- treated, sterile w/lid	Microplate	√
	353280	384-well Microplates, black/clear, BD Optilux, TC- treated, sterile w/lid	Microplate	√
	353284	384-well Microplates, black/clear, BD Optilux, TC- treated, sterile w/lid	Microplate	√
	353289	384-well Microplates, clear, TC-treated, sterile with lid	Microplate	√
	353962	384-well Microplates, black/clear BD Optilux, TC-treated, flat-bottom, with lid	Microplate	√
	353963	384-well Microplates, white/clear BD Optilux, TC-treated, flat-bottom, with lid	Microplate	√
	354660	Poly-D-Lysine 384-well Microplates, white/clear	Microplate	√

384-Well Plates:

Brand	Catalog #s	Plate Name	Tray Type	HD?
BD Falcon	354662	Poly-D-Lysine 384-well Microplates	Microplate	√
	354663	Poly-D-Lysine 384-well Microplates, black/clear	Microplate	√
	354664	Collagen I 384-well Microplates, white/clear	Microplate	√
	354666	Collagen I 384-well Microplates	Microplate	√
	354667	Collagen I 384-well Microplates, black/clear	Microplate	√
	354831	Collagen I 384-well Microplates, clear	Microplate	√
	354832	Collagen I 384-well Microplates	Microplate	√
	354833	Collagen I 384-well Microplates, white/clear	Microplate	√
	354835	Poly-D-Lysine 384-well Microplates, clear	Microplate	√
	354836	Poly-D-Lysine 384-well Microplates, black/clear	Microplate	√
	354837	Poly-D-Lysine 384-well Microplates, white/clear	Microplate	√
	356660	Poly-D-Lysine 384-well Microplates, white/clear	Microplate	√
	356662	Poly-D-Lysine 384-well Microplates	Microplate	√
	356663	Poly-D-Lysine 384-well Microplates, black/clear	Microplate	√
	356664	Collagen I 384-well Microplates, white/clear	Microplate	√
	356666	Collagen I 384-well Microplates	Microplate	√
	356667	Collagen I 384-well Microplates, black/clear	Microplate	√
	356694	Poly-D-Lysine 384-well Microplates, white/clear	Microplate	√
	356696	Poly-D-Lysine 384-well Microplates, clear	Microplate	√
	356697	Poly-D-Lysine 384-well Microplates, black/clear	Microplate	√
	356704	Collagen I 384-well Microplates	Microplate	√
	356705	Collagen I 384-well Microplates, black/clear	Microplate	√
	356831	Collagen I 384-well Microplates, clear	Microplate	√
	356832	Collagen I 384-well Microplates, black/clear	Microplate	√
	356833	Collagen I 384-well Microplates, white/clear	Microplate	√
	356835	Poly-D-Lysine 384-well Microplates	Microplate	√
	356836	Poly-D-Lysine 384-well Microplates, black/clear	Microplate	√
	356837	Poly-D-Lysine 384-well Microplates, white/clear	Microplate	√
	356931	Collagen I 384-well Microplates, clear	Microplate	√
	356932	Collagen I 384-well Microplates	Microplate	√
	356933	Collagen I 384-well Microplates, white/clear	Microplate	√
	356935	Poly-D-Lysine 384-well Microplates, clear	Microplate	√
	356936	Poly-D-Lysine 384-well Microplates, black/clear	Microplate	√
	356937	Poly-D-Lysine 384-well Microplates, white/clear	Microplate	√
Corning	3662	Corning® 384 Well Clear Flat Bottom Polystyrene Poly-D-Lysine Coated Microplates	Microplate	√
	3663	Corning® 384 Well Flat Clear Bottom White Polystyrene Poly-D-Lysine Coated	Microplate	√
	3664	Corning® 384 Well Flat Clear Bottom Black Polystyrene Poly-D-Lysine Coated	Microplate	√
	3683	Corning® CellBIND® 384 Well Flat Clear Bottom Black Polystyrene Microplates, Sterile	Microplate	√
	3701	Corning® 384 Well Clear Flat Bottom Polystyrene TC-Treated Microplates, Sterile	Microplate	√
	3707	Corning® 384 Well Flat Clear Bottom White Polystyrene TC-Treated Microplates	Microplate	√
	3712	Corning® 384 Well Flat Clear Bottom Black Polystyrene TC-Treated Microplates	Microplate	√
	3985	Corning® 384 Well Optical Imaging Flat Clear Bottom Black Polystyrene TC-Treated	Microplate	√
Evotec/PerkinElmer/Greiner	781058	384-well Evotec µClear [PerkinElmer]	Microplate	√
Greiner	781165	Cellstar 384W Plate, PS,F-Bottom, CLR, ST, 10 PCS/Bag	Microplate	√
	781091	CellStar, 384W Plate, PS, TC, ST, BLK/uCLR, Lid 127,8/86 TC, W/LID	Microplate	√
	781092	CellStar, 384W Plate, PS, TC, ST, BLK/uCLR 127,8/86/15 MM, TC,STERILE	Microplate	√
	781093	CellStar, 384W Plate, PS, TC, ST, WHT/uCLR 127,8/86/15 MM,TC, STERILE	Microplate	√
	781098	CellStar, 384W Plate, PS, TC, ST, WHT/uCLR, Lid 127,8/86 MM TC, W/LID	Microplate	√
	781182	CellStar, 384W Plate, PS, TC, ST, CLR, Lid 8 PCS/BAG, W. LID	Microplate	√
	789071-G	Black-wall, uclear from GNF	Microplate	√
Matrical	MGB101-1-2-LG	Black-Wall Glass Bottom MatriPlate 0.17mm	Microplate	√
	MGB101-1-2-LG-L	Black-Wall Glass Bottom MatriPlate 0.17mm with Lid	Microplate	√
	MGB101-1-2-LG-CC-L	Black-Wall Glass Bottom MatriPlate 0.17mm Collagen with Lid	Microplate	√
	MGB101-1-2-LG-PDL-L	Black-Wall Glass Bottom MatriPlate 0.17mm PDL with Lid	Microplate	√
	MGB101-1-2-LG-FN-L	Black-Wall Glass Bottom MatriPlate 0.17mm Fibronectin with Lid	Microplate	√
	MGB101-2-2-LG	White-Wall Glass Bottom MatriPlate 0.17mm	Microplate	√
	MGB101-2-2-LG-L	White-Wall Glass Bottom MatriPlate 0.17mm with Lid	Microplate	√
	MGB101-2-2-LG-CC-L	White-Wall Glass Bottom MatriPlate 0.17mm Collagen with Lid	Microplate	√
	MGB101-2-2-LG-PDL-L	White-Wall Glass Bottom MatriPlate 0.17mm PDL with Lid	Microplate	√
	MGB101-2-2-LG-FN-L	White-Wall Glass Bottom MatriPlate 0.17mm Fibronectin with Lid	Microplate	√
	MGB101-1-1-LG	Black-Wall Glass Bottom MatriPlate 0.72mm	Microplate	√
	MGB101-1-1-LG-L	Black-Wall Glass Bottom MatriPlate 0.72mm with Lid	Microplate	√
	MGB101-1-1-LG-CC-L	Black-Wall Glass Bottom MatriPlate 0.72mm Collagen with Lid	Microplate	√
	MGB101-1-1-LG-PDL-L	Black-Wall Glass Bottom MatriPlate 0.72mm PDL with Lid	Microplate	√
	MGB101-1-1-LG-FN-L	Black-Wall Glass Bottom MatriPlate 0.72mm Fibronectin with Lid	Microplate	√
	MGB101-2-1-LG	White-Wall Glass Bottom MatriPlate 0.72mm	Microplate	√
	MGB101-2-1-LG-L	White-Wall Glass Bottom MatriPlate 0.72mm with Lid	Microplate	√
	MGB101-2-1-LG-CC-L	White-Wall Glass Bottom MatriPlate 0.72mm Collagen with Lid	Microplate	√
	MGB101-2-1-LG-PDL-L	White-Wall Glass Bottom MatriPlate 0.72mm PDL with Lid	Microplate	√
	MGB101-2-1-LG-FN-L	White-Wall Glass Bottom MatriPlate 0.72mm Fibronectin with Lid	Microplate	√

384-Well Plates:

Brand	Catalog #s	Plate Name	Tray Type	HD?
Matrix	4313	384-Well, Clear, Flat Bottom, Tissue Culture Treated, Sterile	Microplate	√
	4329	384-Well, White/Clear Bottom, Flat Bottom, Tissue Culture Treated, Sterile	Microplate	√
	4330	384-Well, White/Clear Bottom, Flat Bottom, Tissue Culture Treated w/ lid, Sterile	Microplate	√
	4331	384-Well, Black/Clear Bottom, Flat Bottom, Tissue Culture Treated, Sterile	Microplate	√
	4332	384-Well, Black/Clear Bottom, Flat Bottom, Tissue Culture Treated w/ lid, Sterile	Microplate	√
	4336	384-Well, Clear, Flat Bottom, Tissue Culture Treated w/ lid, Sterile	Microplate	√
Perkin Elmer	6007430	CellCarrier-384, 384-well microplate, black, clear bottom, TC-treated, sterile, 40/box	Microplate	√
	6007431	CellCarrier-384, 384-well microplate, black, clear bottom, TC-treated, sterile, 5/box	Microplate	√
	6007434	CellCarrier-384, 384-well microplate, black, clear bottom, TC-treated, sterile, 160/box	Microplate	√
	6007440	CellCarrier-384, 384-well microplate, black, clear bottom, Collagen, sterile, 32/box	Microplate	√
	6007441	CellCarrier-384, 384-well microplate, black, clear bottom, Collagen, sterile, 3/box	Microplate	√
	6007450	CellCarrier-384, 384-well microplate, black, clear bottom, Poly-D-Lysine, sterile, 32/box	Microplate	√
	6007451	CellCarrier-384, 384-well microplate, black, clear bottom, Poly-D-Lysine, sterile, 3/box	Microplate	√
Nunc	142761	384-Well Optical Bottom Plates, Black/Clear, with Lid	Microplate	√
	142762	384-Well Optical Bottom Plates, White/Clear, with Lid	Microplate	√
	152029	384-Well Optical Bottom Plates, Black/Clear, Poly-D-Lysine	Microplate	√
	152041	384-Well Optical Bottom Plates, Black/Clear, Collagen I	Microplate	√
	176751	384-Well Optical Bottom Plates, Black/Clear CC3	Microplate	√
	176752	384-Well Optical Bottom Plates, White/Clear CC3	Microplate	√
Porvair Sciences	311003	White, Tissue Culture Treated 384-well Krystal Microplate with lid	Microplate	√
	311503	White, Tissue Culture Treated 384-well Krystal Microplate with lid	Microplate	√
	312003	Black, Tissue Culture Treated 384-well Krystal Microplate with lid	Microplate	√
	312030	Black, Tissue Culture Treated 384-well Krystal Microplate with lid	Microplate	√
	312503	Black, Tissue Culture Treated 384-well Krystal Microplate with lid	Microplate	√
	313030	White, Tissue Culture Treated 384-well Krystal Microplate with lid	Microplate	√

35mm Dishes:

Brand	Catalog #s	Dish Name	Tray Type
BD Falcon	353001	35 x 10mm Easy-Grip Cell Culture Dish, tissue-culture treated polystyrene	21
Corning	430165	Corning® 35mm TC-Treated Culture Dish	21
IBIDI	81151	µ-Dish 35mm, high, hydrophobic, uncoated, sterile	21
	81156	µ-Dish 35mm, high, ibiTreat, tissue culture treated, sterile	21
Greiner	627160	CELLSTAR® Dish, PS, 35x10mm, 8.7cm², ST, TC, Vented	21
MatTek	P35G-1.0-14-C	35 mm Glass Bottom Culture Dish, Uncoated, No 1.0 glass thickness, 14mm glass diam.	21
Nunc	150318	35 x 10mm Dishes Nunclon™Δ Polystyrene.	21
	153066	35 x 10mm Dishes Nunclon™Δ Polystyrene with Airvent.	21
Sarstedt	83.1800.003	SUREGrip 35 x 10mm Cell Culture Dish	21
TPP	93040	35dia x 11mm TC Treated Dish, Growth Area 9.2cm^2	21
WPI	FD35-100	35mm FluoroDish Glass Bottom Sterile Culture Dish	21
	FD35COL-100	35mm FluoroDish Glass Bottom Sterile Culture Dish - Collagen Coated	21
	FD35PDL-100	35mm FluoroDish Glass Bottom Sterile Culture Dish - Poly-D-Lysine Coated	21

60mm Dishes:

Brand	Catalog #s	Dish Name	Tray Type
BD Falcon	353002	60 x 15mm Cell Culture Dish, TC-treated Polystyrene	19
	353004	60 x 15mm Easy-Grip Cell Culture Dish, TC-treated Polystyrene	19
Corning	430166	Corning® 60mm TC-Treated Culture Dish	19
Greiner	628160	CellStar Dish, PS, 60x15mm, 21cm², ST, TC, Vented	19
Nunc	174888	60 x 15mm Dishes Nunclon™Δ Polystyrene.	19
Sarstedt	83.1801.003	SUREGrip 60 x 15mm Cell Culture Dish	19
TPP	93060	60dia x 16mm TC Treated Dish, Growth Area 22.1cm^2	19

100mm Dishes:

Brand	Catalog #s	Dish Name	Tray Type
BD Falcon	353003	Stand Dish Style, Standard TC	9
	353803	Standard Dish Style, Primaria TC	9
	354450	Collagen I 100 mm Culture Dishes	9
	354451	Fibronectin 100 mm Culture Dishes	9
	354452	Laminin 100 mm Culture Dishes	9
	354453	Collagen IV 100 mm Culture Dishes	9
	354455	Poly-D-Lysine/Laminin 100 mm Culture Dishes	9
	354469	Poly-D-Lysine 100 mm Culture Dishes	9
	354600	BD Biocoat Matrigel Matrix Thin Layer 100 mm Culture Dishes	9

100mm Dishes:

Brand	Catalog #s	Dish Name	Tray Type
BD Falcon	354634	Matrigel Matrix 100 mm Culture Dishes for Hepatocytes	9
	354653	Gelatin 100 mm Culture Dishes	9
	356450	Collagen I 100 mm Culture Dishes	9
	356469	Poly-D-Lysine 100 mm Culture Dishes	9
	356653	Gelatin 100 mm Culture Dishes	9
Corning	3262	Corning® 100mm Ultra Low Attachment Culture Dish	9
	3296	Corning® CellBIND® Surface 100mm Culture Dish	9
	430167	Corning® 100mm TC-Treated Culture Dish	9
	430293	Corning® 100mm TC-Treated Culture Dishes in 6-pack Carriers	9
Greiner	664160	CellStar Dish, PS, 100x20mm, 58cm^2, TC, ST, Vented	9
	664940	CellCoat Dish, PS, 100x20mm, 58cm^2, w/PDL, ST	9
	664950	CellCoat Dish, PS, 100x20mm, 58cm^2, w/COLL, ST	9
Nunc	150350	Dishes Nunclon™Δ Polystyrene.	9
	150679	Dishes Nunclon™Δ Polystyrene.	9
	172958	Dishes Nunclon™Δ Polystyrene.	9

150mm Dishes:

Brand	Catalog #s	Dish Name	Tray Type
Corning	430599	Culture Area: 148cm^2; Dia. x H^A: 150 dia. x 25mmH	11
Greiner	639160	CellStar Dish, PS, 145x20mm, 141cm^2, ST, TC, Vented 145,0/20 MM	11
Nunc	168381	150 x 20mm Cell Culture Dish, Lid, With Vent, Sterile	11
TPP	93150	150dia x 20mm TC Treated Dish, Growth Area 147.8cm^2	11

Slides:

Brand	Catalog #s	Slide Name	Tray Type
BD Falcon	354629	BD BioCoat™, Poly-D-Lysine 2-well CultureSlides	Microslide
Essen	9500-4380	FLR Calibration Slide	Microslide
Nunc	154534	Lab-Tek II^{TM} Chamber Slide System, 8-Well Configuration	Microslide

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